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PHILADELPHIA

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GERM CELLS OF COELENTERATES

VI. GENERAL CONSIDERATIONS, DISCUSSION, CONCLUSIONS

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THIRTY FIGURES (THREE PLATES)

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I. INTRODUCTION

The study of the germ cells of the Coelenterates was undertaken with the primary aim of securing a series of observations upon the behavior of these cells in a variety of types within the phylum. The original plan included the study of representative forms of Hydrozoa, Scyphozoa, and Actinozoa. The last class

has been omitted from consideration because of a failure to secure adequate material, but considerable numbers of the other two classes have been carefully investigated.

The earlier studies (G. T. Hargitt, '09 to '18) presented the data obtained from the investigation of particular species, with some discussion of a general sort in interpreting these observations. It is now necessary to consider the observations in the light of the accumulated knowledge of the various species and to correlate the data obtained from different species. It will also be well to discuss the results of these studies in connection with observations upon the germ cells of other phyla. Certain phases of the problem have been reinvestigated and new data obtained; the results of this new study will be considered in place under the appropriate headings.

II. ORIGIN OF THE GERM CELLS

1. Place of origin

The generalization was made many years ago that the germ cells of Hydrozoa always arose from the ectoderm, while in Scyphozoa it was the entoderm which gave rise to germ cells. So far as my observations go, the latter statement is confirmed, but the former is not correct. The genera and species of Hydrozoa which have been investigated are sufficient in number to show that neither the ectoderm nor the entoderm may be considered as the characteristic place of germ-cell origin; on the contrary, these cells may arise sometimes from one layer and sometimes from the other, even in the same species.

A survey of the available literature of recent years on the germ cells of Hydrozoa gave the following results: All who have worked upon Hydra agree upon the ectodermal origin of the germ cells. Thirteen authors record twenty-three other species of Hydrozoa as producing germ cells in the ectoderm and nine authors record thirty-one species in which the germ cells arise in the entoderm. In many cases from two to four authors have studied the same species, in other cases only a single study has been made of a species. The summary made above includes

every record, which means that a few species have been recorded twice when two authors differ in their results. If these disputed cases were omitted, the ratio would remain practically unchanged. Four investigators, working upon six species, agreed that the germ cells might take their origin either in the ectoderm or in the entoderm; Goette ('07) found five species in which the male germ cells were formed in one layer and the female cells in the other; in fourteen species the two sexes agreed in the place of germ cell origin. Other authors have recorded for single species a different place of origin for the two sexes.

Those recent investigators who have studied the Hydrozoa most carefully and extensively are in agreement upon the lack of definiteness in the place of germ-cell origin. They agree that the portion of the polyp or colony where germ cells arise is not always the same, the layer may differ in the same species and in the two sexes of the same species, and they also agree in dismissing the place of origin in germ cells of Hydrozoa as of no significance. The work of the author is in harmony with this opinion.

2. Time of origin

The investigation of the precise time in ontogeny at which germ cells arise comes within the scope of cytological study, rather than in earlier embryological investigation. This change of attitude has developed largely as a consequence of the interest in the germ-plasm theory of Weismann; it is of much importance to the theory to determine the time at which germ cells are differentiated and especially to discover their relation to the fertilized ovum. The studies of Weismann ('83) upon the origin of sex cells of Hydromedusae furnished him with the chief material upon which to formulate his theory. The actual observations of Weismann, did not, in fact, warrant the enunciation of this theory, as has been clearly pointed out by Goette ('07), C. W. Hargitt ('11), the author and others. It is only necessary to refer to the preceding section to note the extent to which Weismann's claim of the ectodermal origin of the germ cells in all Hydrozoa is incorrect; indeed Weismann's own published papers

demonstrate that he often found the germ cells to be first recognizable in the entoderm. The suggestion of an ectodermal origin was proposed on theoretical grounds. But while the ectodermal origin of the germ cells is proved not to be characteristic of Hydrozoa, this does not necessarily discount the germ-plasm theory. If it could be shown that the germ cells arise very early in ontogeny and remain distinct and unchanged to the time of sexual maturity and the formation of the gonads, it would be a matter of no importance where these cells were located in the interim, provided they remained passive and took no part in the functioning of the body.

In certain phyla considerable success has attended the investigation of germ-cell origin; an early differentiation has been noted and these cells have been followed to their position in the gonads. Some of these cases will be discussed later. In Hydrozoa, on the contrary, there has been an almost universal failure to observe the differentiation at any time before sexual maturity was reached. Weismann's studies were made on mature hydroids and medusae, and only as a theoretical suggestion was an early differentiation urged. Harm ('02), in young hydranths of Clava squamata, just developing from planulae, found certain cells which he believed to be primordial germ cells. These cells, figured and described by Harm, are ectodermal cells similar in form, size, and position to the interstitial cells; somewhat later they form elongated, spindle-shaped cells lying directly against the supporting membrane, and possessing a slightly more deeply staining cytoplasm. They were not traced beyond this stage. Wulfert ('02) traced the development of Gonothyraea loveni to the formation of the polyp. While the planula is still within the gonophore, interstitial cells are produced in the ectoderm and entoderm, and these were followed through their differentiation into ganglion cells and nematocysts. After the planula has begun to transform into the polyp, Wulfert finds, for the first time, what he believes to be germ cells. These occur in both ectoderm and entoderm and, according to his figures, are like those cells of an earlier period which became ganglion cells. Furthermore, the cells called germ cells differ from other interstitial cells of all stages only in their staining reaction.

Harm does not describe the formation of ganglion or nettling cells in Clava, but his germ cells follow the same course as the ganglion cells of Wulfert in Gonothyraea, and Wulfert never refers to the spindle-shaped cells as germ cells. It seems clear that both these investigators are dealing with interstitial cells. Wulfert's results would suggest that what Harm called germ cells were in reality differentiating ganglion cells. Wulfert states that his primordial germ cells arise from interstitial cells, but the evidence he presents in favor of considering these as germ cells is not convincing. The determining characteristic, to him, is the more deeply staining cytoplasm, and this, I believe, cannot be considered a sufficient criterion, as I have pointed out in another place (G. T. Hargitt, '16).

Stschelkanowzew ('06) describes germ cells as present in late cleavage stages of Cunina proboscidea. While the embryo is a solid mass of cells and the ectoderm and entoderm are being separated as layers of a single cell in thickness, he finds one or two cells between the ectoderm and entoderm layers, but neither in description nor in figures does he specify the characteristics of these cells. Their size, form, color reaction, and the size of nucleus seem to be the same for the ectoderm cells, the position alone is different. In this instance, also, we have to do either with the formation of interstitial cells or with the completion of the formation of the cells of the central solid mass. Precisely the same process will presently be described for the formation of embryos of Tubularia.

There is no question of the early formation of interstitial cells; these have been found, described, and their differentiation followed in Hydrozoa by various authors. For example, Schneider, ('90) noted the characteristic and early appearance of interstitial cells in Hydra, and their later transformation into ganglion cells, nematocysts, and germ cells. Morganstern ('01) traced the development of Cordylophora through the larval period, and identified the ganglion and nettling cells produced from interstitial cells, but did not find any evidence of germ cells in larvae or young polyps. The germ cells arose from ectodermal interstitial cells at the time of sexual maturity. Schneider and Mor-

ganstern find, what is probably more or less universal in hydroids, that some of the interstitial cells remain undifferentiated for a long time. But such undifferentiated cells are not germ cells, since they form nettling cells throughout the life of the polyp and probably act as replacing cells for any of the epithelial cells destroyed.

In order to test further this question of the presence of germ cells in embryos, I have made a careful, extensive, and entirely new study of the cleavage stages and planulae of Campanularia flexuosa and Gonothyraea loveni; also a similar study of cleavage, embryo, and young polyp (actinula) of Tubularia crocea. In this investigation I have followed the formation of the germ layers, the differentiation of interstitial cells, and especially have searched for primordial germ cells.

In Campanularia and Gonothyraea cleavage results in the formation of a solid morula composed of yolk-laden cells whose boundaries are made out with great difficulty, if at all. The outer cells of the morula arrange themselves into an indefinite ectodermal layer, and later the cells of the solid central mass pull apart to form an enteron, but during this time none of the cells take on a columnar form and no interstitial cells are present. Figure 1 shows the appearance of the embryo after the formation of the enteric cavity; the cells are not sharply outlined, and the nuclei, surrounded by masses of cytoplasm, are irregularly scattered through both the outer and inner layers. This rather indefinite condition is replaced in young planulae by the condition shown in figure 2. The ectoderm cells are now columnar and a few interstitial cells are present, the cells of the entoderm are assuming a columnar form, and deep in this layer are groups of interstitial cells. The boundary between the primitive germ layers is not a definitely formed supporting lamella, but only the cell outlines of the ectoderm. One is immediately struck by the appearance of some of the interstitial cells of the entoderm, and there is little doubt that some of these are similar to the primordial germ cells of Wulfert. However, some of these are spindle-shaped or stellate in form and their nuclei do not differ from the nuclei of the epithelial cells of the entoderm. As the

planula develops, these interstitial cells divide to produce such groups as the one shown in figure 3; at the same time the entoderm cells assume a definite epithelial form. During the progress of the development of the planula the entodermal interstitial cells decrease in number, nematocysts are formed from some of them, and others become elongated, as shown in figure 4. Some of these spindle-shaped cells extend, or move, toward the free surface of the entoderm and have the form and appearance of gland cells. None of the entodermal interstitial cells remain in the form shown in figure 2, and none of them, in the older planulae, display the characteristics of germ cells. During these changes in the entoderm there are very few ectodermal interstitial cells produced (none were present in the region of the planula from which the figure was made), and through the entire history of the planula there are no germ cells in the ectoderm.

Thus, by the time the planula has been perfected, there are no cells in the ectoderm or entoderm which have even the remotest resemblance to germ cells. The almost complete absence of interstitial cells from both germ layers of the completed planulae, and the formation of nematocysts from most of these, renders it certain that primordial germ cells are not present at this stage. Consequently, the cells which resemble those interpreted as germ cells by Wulfert and Harm are not such, but differentiate into specialized cells of the body.

There are some differences in the formation of the morula in Gonothyraea loveni, but once the solid mass of cells is produced the development is so similar to that of Campanularia it has not been thought necessary to describe and figure this form. But it may be said that at no stage could I find even a single cell in Gonothyraea which showed the characteristics of a germ cell. I am forced to believe, therefore, that Wulfert described as germ cells merely interstitial cells which were undergoing differentiation into ganglion cells, gland cells, or some other specialized cell element. Certainly, if primordial germ cells were characteristically present, one should be able to find them, but this study of similar stages of the same species on which Wulfert worked gave no evidence of their presence.

A similar solid morula is produced by the cleavage of the egg of Tubularia crocea, but the cell outlines are sharply defined and the formation of the germ layers is easily followed. During the separation of an outer ectoderm from the superficial cells of the morula, divisions take place (fig. 5) in such a fashion as to result in the production of interstitial cells. But interstitial cells are also formed by divisions from the deeper cells which make up the entoderm (fig. 6). In this early stage the interstitial cells may, but not always do, show a more deeply staining cytoplasm. The ectodermal layer becomes more distinct, its cells become more columnar, and the interstitials increase in numbers to produce the appearance represented in figure 7. The groups of interstitial cells are formed by divisions of the earlier cells, but others are also formed from the cells of the outermost layers. By the time a cavity is present in the center of the embryo the ectoderm has become separated by a supporting layer (fig. 8). At this stage the interstitial cells are numerous, so closely packed as to render their outlines indistinct, and for the most part there is no difference in the staining reaction of the ectodermal and interstitial cells. From this embryonic condition the young polyp or actinula develops. In the development of the polyp the interstitial cells shown in figure 8 are easily followed through their differentiation into nematocysts and other specialized cells. At no time can cells be found which resemble germ cells. This conclusion is in harmony with the earlier results of the author ('09) on this species, for it was then found that germ cells first became differentiated in the medusoid buds of the hydroid from the ectodermal cells.

Other hydroids, as Clava, Hybocodon, Eudendrium, were examined in cleavage and larval stages, but the material was not sufficient in amount to permit a determination of the details noted for Tubularia, Campanularia, and Gonothyraea. No evidences of germ cells were seen in these stages of the forms mentioned. A study of Clava and Eudendrium was made by C. W. Hargitt ('04 b, '06), and the formation of germ layers and interstitial cells was determined. In neither did a differentiation of germ cells occur in the planulae or earlier stages. Other inves-

tigators who have studied the development of Hydrozoa and Scyphozoa record the formation of germ layers and interstitial cells and the differentiation of the latter into ganglion cells and nematocysts, but have not observed the presence of germ cells in these earlier stages.

We may conclude that Wulfert and Harm made no mistake in their observations, but the interpretation of certain cells as germ cells is not justified by their own evidence, nor is it confirmed by this new study. The cells described as germ cells are interstitial cells which were in the process of differentiation into specialized cells of the body. In all the forms carefully studied it is clear that germ cells do not occur in larvae or young polyps, and in the absence of any evidence of their presence in similar stages of other forms, there is ample reason for concluding that an early differentiation of germ cells does not occur.

The germ cells of Hydra have been investigated by a considerable number of investigators, and practically all of these agree upon the origin from interstitial cells of the ectoderm at the breeding season. Brauer ('91) has observed the formation of the interstitial cells before the ectoderm and entoderm are fully separated, and has followed the differentiation of these into ganglion cells and nematocysts, confirming the earlier results of Schneider ('90). Downing ('05, '09) is the only one who has suggested a different conclusion for the germ cells of Hydra. He observes the same origin from interstitial cells, but, in the developing ovary, finds some interstitials to be larger than others; these he believes to be primordial germ cells which have been segregated in early ontogeny to form a 'self propagating' germinal tissue. He has not observed these cells in the embryo, indeed he seems to have studied only the polyps which are producing reproductive organs, and therefore his conclusions are largely hypothetical. The presence of larger interstitial cells in the developing ovary and their identification as germ cells is confirmed by Tannreuther ('08), who also finds similar cells forming spermatogonia. But in every case the formation of ovaries and spermaries is initiated by a rapid growth of interstitial cells and later a multiplication of these cells. Tannreuther thus accounts for the presence of larger

interstitial cells, but shows they are not a germinal tissue. He finds no such cells before or after the formation of the reproductive organs and can trace their growth from ordinary interstitial cells. Later, Tannreuther ('09) followed the behavior of the interstitial cells and could find all gradations between small and large interstitials, as well as trace the transformation of an ordinary interstitial through spermatogonia into spermatozoa.

Wager ('09) finds no evidence of a germinal tissue in Hydra, nor of any difference between interstitial cells. Furthermore, in the very groups of interstitial cells which grow to form oogonia, "one usually finds nematocysts developing in large numbers. In the course of development of the ovarian area these nematocysts either migrate out or are resorbed. Frequently they are found within the egg itself." This is a very striking demonstration of the equipotency of the interstitial cells and effectually refutes the belief of a distinct germinal tissue composed of certain interstitial cells. The characters used by Downing to differentiate germ cells from other interstitials are found to be applicable to most interstitial cells; there is great variety in size and appearance, and Wager finds all gradations between these variations in interstitial cells. He strongly confirms the work of the earlier authors and agrees perfectly with Tannreuther in the absence of a distinct germinal tissue in Hydra. The work of these two authors did not include a complete study of the histogenesis, but the investigations of Schneider ('90) and Brauer ('91) completely fill this gap. Hegner ('14), in discussing the germ cells of Hydra, says he "is inclined to accept Downing's position in the matter." But Downing's position is untenable, for his conclusions are refuted by the work of other investigators. The strongest evidence of the occurrence of a distinct germinal tissue presented by Downing, viz., the presence of larger interstitial cells of a distinct sort, is shown by both Wager and Tannreuther to be merely an incident in the formation of reproductive organs.

From the facts presented in the above discussion there is but one conclusion which may fairly be drawn, viz., in Hydra and other Hydrozoa there is no clear evidence that germ cells are ever differentiated in larvae, young polyps, or any early stage in ontogeny.

In addition to the line of evidence just presented, we have direct observations upon the entire germ-cell cycle of some hydroids from their earliest differentiation. Some of the thirty or more species studied by Goette ('07) give very clear evidence of the method of origin of germ cells. In Podocoryne germ cells arise from both ectoderm and entoderm; in Corydendrium parasiticum the egg cells are formed from ordinary entoderm cells by division, a basal egg cell and a distal epithelial cell resulting, and only this method of formation is applicable in this species. A similar division takes place in Clava, and Goette says, "no doubt exists, that the egg cells of Clava multicornis proceed only from transformed half entoderm cells." He observed epithelial cells dividing, one half forming the egg cells of Sertularia argentea, Gonothyraea loveni, Obelia longissima, and the sperm cells of Eudendrium. In Obelia geniculata the eggs develop only in the medusae by the transformation of entire entoderm cells. Smallwood ('09) traced the egg cells of Hydractinia echinata back to single entoderm cells which underwent no division, but transformed directly into oocytes. Campanularia flexuosa produces its eggs by a similar transformation of entire entodermal epithelial cells or from the basal half of a divided entoderm cell, the distal end of which persists as an epithelial cell (G. T. Hargitt, '13). The author ('16) also observed egg cells typically arising from half entoderm cells in Clava leptostyla, though occasionally from ectodermal interstitial cells.

Such observations upon a number of species by different investigators leave no doubt of the entire normality of the described transformation of tissue cells into germ cells. In such cases there can have been no differentiation and segregation of germ cells in the early ontogeny, for they came from functional tissue cells, a portion of which continued as a tissue cell. Such a cell is a specialized cell and not a latent germ cell. In certain Hydrozoa, therefore, the origin of germ cells has been precisely determined and an early differentiation shown to be impossible; in the absence of positive evidence to the contrary, it would probably be fair to believe that none of the Hydrozoa show a differentiation of germ cells till sexual maturity approaches.

3. Conclusions

In the last twenty years the reports of investigations upon the origin of germ cells of Hydrozoa show more species in which such cells proceed from the entoderm than from the ectoderm. Numerous cases are recorded in which the place of origin differs in the sexes of a single species and where the same individual may produce germ cells from different layers. Furthermore, the germ cells come from different sorts of cells. All of this points to the conclusion that the place of origin is variable and not a matter of any significance. A few cases are reported of the origin of germ cells in the embryos or larvae of Hydrozoa, but new investigation of these gives no confirmation of this. Interstitial cells are differentiated in early ontogeny and undergo early specialization into ganglion, nettling, and other cells, but those not so specialized are alike in all respects and at most persist as somewhat inactive cells. During all the life of the polyps these produce nettling cells, form replacing cells, and, in some species, at sexual maturity produce germ cells.

In none of the Hydrozoa has the differentiation of germ cells been demonstrated in early ontogeny. On the other hand, observations of several species have demonstrated that germ cells may arise from body cells directly, either by the transformation of an entire cell or from the transformation of one half of such a body cell. Obviously in such cases an early differentiation of germ cells is out of the question, and it is believed to be typical of Hydrozoa to form their germ cells only at the time of sexual maturity.

III. THE GERM-PLASM THEORY

1. General statement and discussion of the theory

This theory has been much discussed and many weighty objections have been raised against it; at the same time it has been strongly defended and important evidence brought forward to uphold it. Probably the lines of defense, as well as of opposition, are so well known as not to require further review. There-

fore, the present discussion will be limited to a consideration of the theory in relation to observed facts in the Hydrozoa. In order to have clearly in mind the essential features of the theory and its method of application to the Hydrozoa attention is directed to the statements of the author of the theory.

In every ontogeny, a part of the specific germ-plasm contained in the parent egg cell is not used up in the construction of the body of the offspring, but is reserved unchanged for the formation of the germ cells of the following generation (Weismann, '91, vol. 1, p. 170).

This splitting up of the substance of the ovum into a somatic half, which directs the development of the individual, and a propagative half, which reaches the germ cells and there remains inactive, and later gives rise to the succeeding generation, constitutes the theory of the continuity of the germ plasm, which I first stated in the year 1885 (Weismann, '04, vol. 1, p. 411).

. . . . In hydroids the germ cells do not appear in the 'person' which is developed from the ovum at all, and only arise in a much later generation, which is produced from the first by continued budding. . . . . In all the last mentioned cases the germ cells are not present in the first person arising by embryogeny as special cells, but are only formed in much later cell generations from the offspring of certain cells of which this first person was composed. These ancestors of the germ-cells cannot be recognized as such: they are somatic cells—that is to say, they, like the numerous other somatic cells, take part in the construction of the body, and may be histologically differentiated in different degrees (Weismann, '93, p. 185).

Invisible, or at any rate unrecognizable, masses of unalterable germ-plasm must have been contained in the body cells in all cases in which such a transformation has apparently occurred (Weismann, '93, p. 19).

In the hydroids, then, Weismann notes the germ cells as unrecognizable till the period of maturity; their origin at that time is from body cells which are morphologically differentiated and physiologically specialized to perform certain functions of the animal. This is a statement of fact which is confirmed by the work of the authors referred to in section II of this paper. These facts do not fall into line sufficiently with the theory as stated in the first two quotations, and Weismann thereupon assumes the presence of invisible and unalterable determinants which lie latent in the body cells till activated in some way not specified. This point of view is one to which the greatest objection has been raised. Lloyd Morgan ('91), in a very searching analysis

and criticism of this position, points out its weakness and considers the recourse to invisible units as a hindrance and not an assistance to an understanding of the facts. In any effort to test the theory by observed results in hydroids one is met by the distinct statement that when germ cells arise from body cells the latter contain invisible and unrecognizable materials. If the germ plasm be really invisible and unrecognizable, the theory need not be discussed, since it cannot be proved or disproved. In the following pages evidence bearing upon the theory is presented from various lines of investigation, but the point of view is taken that there must be recognizable differences of some sort, or else an unbroken line must be traceable from germ cell to germ cell in the life cycle.

2. Evidence from Hydrozoa

a. Germ cells. The earlier section of this paper upon the origin of the germ cells is pertinent here, and should be considered in its entirety as a part of the evidence. It may be repeated that the facts show an absence of differentiation of germ cells in early ontogeny; an absence of a definite migration and germ-track; and the formation of germ cells at the time of sexual maturity from different layers and cells of the body. It has been possible to trace the germ cells back to tissue cells and observe the method by which they are produced; Weismann's own observations confirm this perfectly. It is even possible to prove that there cannot be present in the body cells which form germ cells any invisible germ-cell determinants. Goette ('07) and the author ('13, '16) find cases where division of a tissue cell results in the formation of two cells, one of which becomes a germ cell while the other persists as an epithelial body cell. If invisible germ plasm be present in the chromatin, as Weismann distinctly states, how is it possible for one of the two cells to become a germ cell and the other a tissue cell when the chromatin is equally divided and none of it lost? This is crucial evidence, and it gives the facts demanded by Weismann himself to prove his contention incorrect, as Goette and the author have already pointed out.

Without repeating all the evidence presented in sections I and II, the facts may be summarized as follows: there is no definite place of origin of germ cells; there is no definite migration of germ cells and no germ-track; there is no invisible germ plasm in the body cells. Not only is there no continuous germ plasm, so far as can be determined by observation, but the evidence is such as to show the absence of invisible germ plasm. Hegner ('14) is willing to admit the germ cells in Coelenterates do not belong to any germ layer, but he maintains that germ cells are present at all times in a dormant condition. This opinion is based upon the conclusions of Downing, Wulfert, and Harm. The error in the interpretation of these authors has been pointed out and consequently the opinion that germ cells are present in a latent condition at all times is no longer tenable; all the facts are inconsistent with this view.

b. Budding. Budding has generally been held to be a process of growth and cell division, often an evagination taking place. But Weismann says, ". . . . I reached the conclusion, that the budding idioplasm, which must be the starting point of the budding process according to my view, could not be divided between both germ layers, but probably was to be found in only certain cells of the ectoderm." At Weismann's suggestion, Lang ('92) undertook to test this hypothesis and studied budding in Hydra and some hydroids. Weismann believes Lang's results ". . . . contain a perfect confirmation of my conjecture that the same [buds] come from the ectoderm and that actually the 'Budding-idioplasm' had its position entirely in the ectoderm cells." These quotations from the preface to Lang's paper show the application made by Weismann of the germ-plasm theory to this form of asexual reproduction. Lang believed his results showed the proliferation of a few ectoderm cells to form a mass from which the ectoderm and entoderm of the bud developed. After the two layers were formed, a cavity was produced in the bud, and this became continuous with the parent enteron. Braem ('94) repeated the work of Lang on the same and other forms, but could not confirm his results; on the contrary, he observed the division of cells in ectoderm, interstitial, and

entoderm, and the participation of all these layers in the formation of the bud by evagination. He says, ". . . . consequently I do not hesitate to proclaim the results of Lang as erroneous, the conclusions drawn from them as utterly false."

Downing ('05) believed sexual and asexual reproduction in Hydra to be mutually exclusive, and implied a relation between budding and germ cells. Montgomery ('06) supposed sexual reproduction to be the more primitive, and asexual reproduction to be a secondarily derived process; for him, regeneration and asexual reproduction were dependent upon the presence of germ cells. R. Hertwig ('06) found budding and sexual reproduction proceeding side by side in Hydra and believed buds were produced by the activity of the cells in all the layers. Mrázek ('07) and Nussbaum ('07) confirm Hertwig on the simultaneous presence of buds and sex organs in Hydra. The view of Hadži ('09) was in partial accord with Weismann and Lang, for he again renewed the claim of the activity of only a certain layer to form buds in Hydra. In his opinion the interstitial cells were the active elements in producing buds, the other layers not participating in any way. According to this view, the interstitial cells are a source of all new growth, differentiation, and development in Hydra, but they do not necessarily form a germinal tissue. Tannreuther ('09) investigated budding still further, and for two species of Hydra found, first, an increase in volume, and then a proliferation of interstitial cells in the budding zone. There was no migration of interstitial cells into the entoderm as Hadži had believed, for the layers remained distinct and unbroken throughout the process. A distinct evagination occurs and cells of all layers divide mitotically and are active in the budding process. Furthermore, the division of cells of the ectoderm and entoderm began about as soon as in the interstitial cells. Tannreuther's work establishes the fact that budding in Hydra is an evagination due to cell multiplication and growth, all layers in the budding zone participating in the process. It seems probable that the earlier division of the interstitial cells is merely an expression of a more prompt response on the part of the indifferent cells than of the specialized ectoderm and entoderm. I believe the fact is

established that budding in Hydra and hydroids is a process of evagination, but the work of Lang, Hadži, and Tannreuther suggests an earlier activity of the interstitial cells. Even if the interstitial cells were entirely responsible for the formation of the bud, proof would not be thereby constituted for the germinal nature of these cells, for they are differentiating into nematocysts throughout the life of Hydra. Also these same cells transform directly into ganglion cells earlier in the life history.

Medusae are sexual individuals and ordinarily reproduce only by eggs and spermatozoa, but there are a considerable number which undergo a process of asexual reproduction and form other generations of medusae by budding. The budded medusae later become mature and form sex cells just as do the parent medusae. The author ('17) has given a detailed account of this secondary budding of medusae and of germ-cell formation in Hybocodon prolifer; the gonads are produced from the ectoderm of the wall of the stomach, while the new medusae come from the tissues of the base of the tentacle at the margin of the bell. In a critical examination of these medusae no evidence was obtained of the migration of germ cells from the old to the budding medusae, but the new buds arose from both layers of cells in the tentacle after these cells had undergone regressive changes and become embryonic. In Hybocodon the asexual budding is not influenced by the formation of sex organs. Müller ('08) is in error in believing the two methods to be mutually exclusive, for C. W. Hargitt ('02), Perkins ('04), and the author ('17) have recorded abundant cases of the simultaneous presence of buds and gonads.

A. Agassiz ('65), Haeckel ('79), C. W. Hargitt ('04), Mayer ('10), and others have described many cases of asexual budding in medusae. Such buds may be formed, a few at a time, or many at a time; a single generation of buds may be produced or many generations; and many regions of the medusae may be concerned in their formation. Haeckel describes the buds on the stomach wall of Sarsia gemmifera (S. siphonophora) (fig. 10), more than twenty being present at one time and several generations being produced; in different species of Cytaeis (fig. 14) enormous numbers of medusae may be budded from the stomach wall at

the same time that gonads are present. Medusae are formed from a single tentacle base in Hybocodon prolifer, Amphicodon amphipleurus Haeckel and others; from the bases of all tentacles in Sarsia codonophora (fig. 13); from radial canals of Proboscidactyla ornata (fig. 9); from the margin of the bell in Niobia dendrotentaculata (fig. 11); and from the gonads of Eucheilota paradoxica (fig. 12) and other forms. These are merely examples of the variation in the method of budding as recorded for numerous medusae. In many of these the budding occurs during the immature period, and only after budding ceases do the gonads form, but others show no such periodicity and may produce buds and germ cells simultaneously.

The production of the buds from the gonads has been critically studied. Mayer ('10) describes this process for Eucheilota (fig. 12) as involving the activity of the tissues of the gonad and of the tissues outside the gonad; both ectoderm and entoderm of the parent take part in the production of the bud by a process of cell multiplication and evagination. In Phialidium mccradyi buds are also produced from the gonads, but only indirectly, since a blastostyle is first formed and from this the medusae arise by budding. Sigerfoos ('93), in the formation of the blastostyle and medusae, discovered no difference from ordinary cases of budding, the ectoderm and entoderm evaginating to produce the new growth. The germ cells in the gonad play no part in the process other than to behave as all other cells of ectoderm and entoderm, which suggests the probability of the germ cells being merely body cells capable of acting with other body cells or undergoing a growth in preparation for sexual reproduction.

Budding in medusae is typically an evagination of the two body layers, irrespective of the part of the animal which produces the bud, but a few medusae are known to form their buds only from the ectoderm. Mayer describes such a case in Bougainvillia niobe, the ectoderm of the stomach wall differentiating to form all the tissues and organs of the bud. Mayer believes a possibility exists of the origin of the bud entoderm from parent entoderm; but could find no evidence of such a connection, nor of any union of the enteric cavities of bud and parent at any stage

of the process. Chun ('95) describes a similar process in Rathkea octopunctata and Lizzia claparedei. He describes the origin of the bud by the proliferation of a group of ectoderm cells which becomes isolated as a definite mass, though still held in place against the stomach wall, from which all organs of the bud are developed. In these forms the enteric cavities of buds and parents later unite. When sexual maturity is reached germ cells are formed in the stomach wall where the bud was developed earlier, but Chun does not consider the budding as due to a geminal process. Rather, he believes the ectoderm and entoderm of the medusae to be alike in histological and organogenetic structure and potency. Braem ('08) reviews and confirms the work of Chun, but finds germ cells are present in the stomach wall at the same time the bud is forming; he believes the group of cells which start the bud are oocytes, and looks upon the budding process as a short and rapid method of producing a new organism out of cells which are germinal in character. Most budding, he believes, shows no relation between bud and sex cells, and in these cases all layers are essential to the formation of the bud because each tissue has retained only the ability to produce cells of its own kind. Mayer thinks Braem has produced strong evidence that this sort of budding is a germinal process, but does not believe the evidence is conclusive. Child ('15) interprets this case as showing both sex cells and asexual buds come from the functional and more or less specialized cells of the parent medusa.

Nekrassoff ('11) studied Eleutheria dichotoma, which produces buds from the outer wall of the ring-canal. In this form budding parallels sexual development, but does not interrupt it, nor is budding interrupted by sexual development. In a single individual one may find numerous buds, young and old, young and old eggs, cleavage stages and young polyps—all at the same time. The budding takes place in the usual way, involving both ectoderm and entoderm, and while Nekrassoff finds conditions which resemble the observations of Chun and Braem in Rathkea and Lizzia, he can demonstrate the continuity of bud and parent tissues at all times. He does note that the ectoderm and entoderm

cells show a more embryonic appearance after they have begun to form the bud than they did before; especially is this true of the entoderm. Nekrassoff concludes: "on the ground of the observations on the budding of Eleutheria we may conclude that in the Coelenterates already differentiated cells have been given the possibility of a reversible process—the possibility of taking on anew an embryonic character." Regarding the suggestion of the origin of buds from germ cells, he finds in Eleutheria no relation at all between sex cells and buds.

The process of budding in medusae does not, as a rule, involve any difference in principle from budding in Hydra and hydroids, since both germ layers, by cell multiplication and evagination, form the outgrowths which, by later differentiation, become the tissues of the new individual. There are some buds which arise from a small group of cells of a single layer, but in no case do buds come from a single cell. Budding is not, therefore, a germinal phenomenon, even when the new growth is derived from the tissues of the gonads. Consequently, not only is there no necessity for thinking of the germ plasm as being essential to the formation of buds, but there is no evidence of the presence of germ plasm in these buds. The conclusion of Nekrassoff, that differentiated cells may take on again an embryonic character, seems to explain the facts better than the germ plasm theory. Though quite unaware of this conclusion of Nekrassoff, the author ('17) worked out the budding of Hybocodon medusae and noted the embryonic character of the cells involved in the budding process.

There is considerable variation in the degree to which this 'reversible process' is exhibited by the tissues of medusae, but an unbroken series may be arranged which includes all the known types of budding. At one end of the series we may place the medusae whose tissues do not have such a capacity; these reproduce only from fertilized egg cells. Here are included the majority of medusae. If we accept the conclusions of Braem, we may next place forms, like Lizzia and Rathkea, in which a group of unfertilized oocytes may develop into a new organism. This is a very unusual method and is applicable, so far as known, only to the two forms named. Here the tissues either have no

power to change or the stimulus to such change would be lacking. Following this would come Bougainvillia niobe; the ability to form buds is limited to a definite tissue, the ectoderm. Next are those forms like Hybocodon in which all layers cooperate to form buds, but this capacity for asexual reproduction is limited to a definite locality in the parent. In this category one would place most of the medusae which form buds, and all hydroids and Hydra. Niobia dendrotentaculata represents a type in which the bud is partly new growth and partly the already formed organs of the parent; presumably all the regions of the body in such forms would have the ability to undergo some transformation. This type of budding would really be intermediate between regular budding and fission. A final group would comprise medusae in which a real fission occurs, and such a method of asexual reproduction is recorded by Mayer ('10, vol. 2, p. 280) for Gastroblasta raffaelei Lang. A gradation such as this would correlate the various kinds and degrees of asexual reproduction in Coelenterates with reproduction in protozoa, with regeneration, and with sexual reproduction. It may even mark a possible evolution of reproductive processes in Coelenterates, but would appear to have no meaning according to the germ-plasm theory.

c. Regeneration. Weismann ('93) takes the position that regeneration is due to the presence of germ plasm, since the latter is the only substance capable of giving rise to all parts of the body. As applied to plants, this involves the presence of germ plasm in the cambium tissue wherever it is found. There is postulated in plants an accessory germ plasm, concerned with the vegetative development, and a primary germ plasm which is retained unchanged till the germ cells are produced. But vegetatively produced buds may later form reproductive organs and cells; this requires the further assumption that accessory germ plasm also contains primary germ plasm. This same involved and intricate explanation is required to account for regeneration in animals, if we believe that regeneration is due to latent germ cells.

Morgan ('01) discusses a considerable number of theories of regeneration and rejects the germ-plasm theory completely, since he finds so many facts of regeneration utterly contradicting

it. He found, for example, that the regenerating organs in annelids came partly from the old organs and partly from new sources; new muscles came, not from old muscle or even from mesoderm, but from the ectoderm, the pharynx regenerated from entoderm instead of ectoderm as in the original development. Other evidence of the same sort was directly contradictory to the view that regeneration is due to latent germ cells. Morgan ('01, '07) believes regeneration is a growth process. Schultz ('02) thinks regeneration is a primary property of life, limited more or less in consequence of specialization of tissues, but always potentially present. His conclusion is in accord with that of Morgan, and implies development, budding, and regeneration to be exhibitions of the capacity for growth inherent in all protoplasm. Montgomery ('06) and Hegner ('14) reject this view and accept the germ-cell explanation, the latter stating that regeneration in Coelenterates is always due to widely distributed germ cells. C. W. Hargitt ('11) points out serious objections to this explanation in hydroids, and Hegner admits the impossibility of accounting for regeneration of sex organs on this view. But sex organs are readily regenerated in hydroids. Child ('15) has observed that specialized cells of Pennaria may undergo a dedifferentiation and take part in budding, along with the interstitial cells; the same thing occurs during regeneration. Morgan has also found abundant evidence of the formation of masses of indifferent cells by regressive changes, and the production of new structures from such masses in regeneration. Morrill ('18), working upon the regeneration of appendages in salamanders, observed the formation of masses of cells by simplification of old specialized cells, and the differentiation of muscle and cartilage from these cells.

In Hydra and hydroids regeneration may take place at practically any point where a cut is made, and almost as often as new growths are excised. Very minute pieces may also regenerate complete animals, normal in all respects, including reproductive organs. The minimal size is always a group of cells, and yet, according to the theory of regeneration from germ cells, there is no reason why a single cell might not produce a new organism,

for the theory supposes the germ cells to be scattered over the whole body in great numbers. Clearly, there is no evidence that regeneration in Coelenterates, nor in other animals, is a process dependent upon the presence of germ cells. And there is abundant evidence that the specialized cells undergo regressive changes, produce masses of cells or syncytia of embryonic character, and then, by differentiation and specialization form new parts to replace those lost. It is, of course, equally well known that not all tissues can undergo such changes or even regenerate their own kind of tissue to any great extent; but this offers no evidence of a correlation between regeneration and the presence of germ cells. It only shows that specialization may proceed to such a degree that further changes, whether progressive or regressive, are impossible.

So far from regeneration presenting evidence in favor of the germ-plasm theory, practically all the experiments and observations show direct contradictions to this explanation. The germ-plasm theory is not only inadequate to explain regeneration, but it is shown to be incorrect, so far as this process is concerned.

d. Dissociated cells. The tissues of sponges have been broken up by teasing and forcing through fine screens, and the behavior of the isolated cells followed by Wilson ('07). Such cells showed amoeboid activities and fused into masses which later regenerated to form normal sponges. The amoebocytes first began to unite to form syncytia, but collar-cells and other specialized cells also took part in the formation of the masses, first passing through a regressive differentiation. Müller ('11) largely confirmed Wilson, but believes such specialized elements as collar-cells do not assist in the regeneration. Fresh-water sponges also undergo normal degeneration phenomena by a de-differentiation of cells to produce embryonic masses which later produce new organisms. This latter process is quite distinct from gemmation.

Later, Wilson ('11) extended his experiments to hydroids. Here also the isolated cells fused into syncytial masses which secreted perisarc about themselves, then formed ectoderm and entoderm layers, and later regenerated hydranths, complete and

normal, with tentacles, mouth, hypostome, and other structures. In these changes "we apparently have . . . . . a plain case of despecialization of tissue elements and their union to form masses of totipotent regenerative tissue." Wilson discusses the question as to whether the tissue cells may not merely retain their specificity and later produce only cells of the same sort. By following the isolated cells with the microscope it was possible to observe the change of the tissue cells from their typical appearance to that of embryonic cells, and their fusion into a mass. The retention of their original specificity seems highly improbable. A histological study of sections of the coalesced cells showed the cells, first, as embryonic in appearance, and, as regeneration proceeds, they undergo changes similar to those seen in normal development and specialization. DeMorgan and Drew ('14), in similar experiments, for the most part confirmed Wilson, but did not obtain hydranths from the regenerating masses. They differ from Wilson in thinking the cells are segregated and rearranged and do not form syncytia by despecialization. They also state their belief that their cell masses are abnormal and pathological, but this does not appear to be the case, as C. W. Hargitt ('15) has pointed out in some detail. This latter author confirmed Wilson's observations in practically every respect, and also noted in detail the behavior of cells immediately after their isolation. The identification of the different cells was easily made, but the characteristic features gradually became less marked and finally disappeared as the cells merged into a common mass. "They have become despecialized into potentially embryonic cells, and probably from this change have acquired their regenerative capacities."

In discussing these experiments, Hegner ('14) claims there are always germ cells present, which would exlain the regeneration from the masses of cells, and therefore a continuity of germ plasm exists in these phyla. He does not attempt to explain the de-differentiation actually observed to occur, though this is a very significant fact and one that cannot be ignored. For, if tissue cells may become embryonic and form other cells and tissues by later differentiation, there is no reason for assuming

the presence of germ cells. The later work on dissociated cells gives clear evidence on this point. DeMorgan and Drew can recognize and follow the isolated ectoderm and entoderm cells and ". . . . in addition such structures as nematocysts, ova and broken down cells, all of which are subsequently absorbed and played no part in the future development." C. W. Hargitt also finds that the presence of germ cells in regenerating masses does not influence the behavior: "Indeed, in those cases in which egg cells were present they took no part whatever in later regenerative activity, either degenerating or being absorbed as yolk material." So far from the regeneration being conditioned upon the presence of germ cells, the latter serve no purpose but to act as food; growth and differentiation are the result of the activity of the tissue cells alone. Since these observations have been confirmed by a number of workers, it is manifestly false to consider regeneration to depend upon germ cells in these plasmodia. There would appear, likewise, to be no ground for assuming any regeneration to be dependent upon germ cells.

The claim of DeMorgan and Drew, of the retention of their distinct structure by the isolated cells, and a later rearrangement to produce the regenerated structures, is not confirmed by any of the other workers. The latter agree in being able to follow the isolated cells through a gradually decreasing sharpness and a final coalescence into a common mass. No doubt occasional cells persist, but the observations clearly show the fusion of the cells into a multinucleate mass. From such a mass a development occurs which parallels the normal development from the egg.

These experiments give such striking and clear-cut results that one is enabled to draw very definite conclusions. Tissue cells have actually been followed through the process of despecialization to an embryonic condition; such embryonic cells behave as any other group of similar cells, and develop a variety of structures which become differentiated and specialized in such a way as to produce a complex, normally organized, and functional individual. The totipotency of the tissue cells of the hydroid is

thereby definitely established, though this is clearly dependent upon the proper stimulus for its exhibition. When we take into consideration, also, the observations upon the origin of germ cells from tissue cells; the observations of Child upon the dedifferentiation of cells in a great variety of animals and their later differentiation into a different sort of cell; the observations upon the formation of embryonic masses from which new structures develop in regenerating worms and salamanders; it would seem as though the germ-plasm theory was the very one of all theories least capable of accounting for the facts.

3. Evidence from other phyla

Such phyla as the round worms and arthropods give the strongest evidence of early segregation of germ cells and the best support of the germ plasm theory. This view is not universally accepted, however, and the opposing opinions are worthy of consideration. For instance, Child ('15) states that it is not known whether the primordial germ cells of Ascaris produce only germ cells or the reproductive organs as well. If the latter be the case, "the germ path of early cleavage has not resulted in the segregation of germ plasm from the soma, but merely in the segregation of different organs," since the walls of the reproductive organs are not germ plasm. The same author points to the fact that in no case is a segregation of germ plasm and soma known to take place at the first cleavage, as the theory requires. He believes, even in these phyla, the theory is unproved, and is not in accord with many facts.

In many animals the germ cells are produced periodically at the breeding season, and at no other period is it possible to recognize germ cells, or even reproductive organs. In these cases the germ cells obviously arise from the tissue cells; it does not answer to claim an invisible germ plasm in the tissue cells, since this is not capable of investigation and evades the question. Other animals are produced asexually and at a later period develop reproductive organs; the germ cells to all appearances, in such cases, come from the more or less differentiated cells of the region involved in the formation of these organs.

In the vertebrates the germ cells appear, as a rule, only after most of the other organs are laid down, and in most cases an early segregation of germ cells has not been proved. A review of the work on vertebrates is given by Hegner ('14) and Kingery ('17), and only a few cases will be mentioned here. Von Winiwarter and Sainmont ('08), from studies upon the cat, describe the degeneration of all the germ cells produced during embryonic development; the definitive eggs arise from the undifferentiated germinal epithelium after birth. Bachman ('14) in Teleosts and Witschi ('14) in Rana temporaria find no evidence of the origin of germ cells from the peritoneum, while v. Berenberg-Gossler ('14) believes "that one may no longer speak of a germ track in the Sauropsida," and Gatenby ('16), in Rana temporaria, observes the majority of germ cells arising from the peritoneum. Kingery ('17), working upon the white mouse, gets results comparable to those of von Winiwarter and Sainmont in the cat; viz., all germ cells formed during the foetal period degenerate and have nothing to do with the development of the definitive ova. The latter arise from the germinal epithelium after birth and all transitional stages between this germinal epithelium and graafian follicles were observed and the development followed.

In the vertebrates and some other phyla the evidence seems to be as clearly opposed to a continuity of the germ plasm as it is in the coelenterates. There is, especially in mammals, an increasing amount of evidence that the germ cells arise from more or less differentiated tissue cells at a time approaching the period of sexual maturity.

4. Evidence from tissue cultures

While most of the experiments dealing with explanted tissues have to do with growth, movements, and general behavior of the cells, there is some evidence of a de-differentiation of the tissues into a more embryonic condition. There is very little evidence that such cells re-differentiate into cells of a new kind, but this return to an embryonic condition resembles somewhat the despecialization of isolated cells of hydroids and sponges.

In cultures of skeletal muscle of chick embryos, Lewis ('17) observed the growth of the cut ends of the muscle into embryonic tissue without striations. Streeter ('17) observed a de-differentiation of cartilage cells in the normal development of the ear in human embryos, the cartilage of the membranous labyrinth undergoing a despecialization and a return to the condition of embryonic connective tissue. From experiments with muscle, kidney, eye, thyreoid, and other organs, Champy ('14) observes a characteristic behavior of the cells of the edge of the culture where they receive abundant air and food. These cells form such an indifferent mass as to resemble cells of a young blastoderm; and this is true for all tissues, irrespective of their source or the culture medium. Such a de-differentiation takes place from explanted adult tissues as well as from embryonic tissues.

Danchakoff ('18) mashes adult spleen and grafts it upon the allantois of embryos. The spleen tissue forms a syncytium of embryonic character, and the cells forming the mass contain endothelial cells of blood-vessels as well as reticular tissue of the spleen. The syncytial mass develops and forms cells of a different sort than those which composed it. Danchakoff interprets this, not as a de-differentiation, but as an expression of an inherent capacity of the original cells to undergo a further differentiation. Her point of view is as follows (p. 161):

> The changes undergone by the living matter during development are not always specific. They may lead to a specialization of tissue without differentiating them specifically. The difference between these two processes consists in that specialization does not imply a limitation of potencies in the cell, while specific differentiation is a process, by which the constitution of a cell is changed irrevocably and its potencies to development are narrowed. The distinction between the two processes would make it unnecessary to introduce a new concept of dedifferentiation in order to understand certain phenomena.

I am not convinced that this view is simpler or more nearly interprets the phenomena observed than the view of regressive changes in the tissues and a later differentiation of these. Nor does this opinion take into consideration the fact that de-differentiation has actually been observed to take place; that is,

specialized cells do actually become embryonic. But for the present discussion the important point is the observation of the varied potencies of the tissues of a differentiated adult organ like the spleen.

This brief account of some of the experimental investigations upon cells and tissues of adult and embryonic animals is enough to show the degree to which such tissues may change their structure and function. It clearly demonstrates that body cells are not so limited in behavior and so predetermined in potency as to render a change impossible. The difference between body cells and germ cells is proved by such investigations not to be so great as is usually held.

5. Evidence from cancer cells

The studies which have been made upon cancers throw some light upon the potencies of tissue cells. As is well known, it is possible to transplant cancers from one animal to another through many generations. Most of the cancers which have been experimentally studied are tissue growths, not germ-cell growths, and the ability of these cells to continue their growth and proliferation for long periods of time is an indication of the ability of tisssue cells to live and grow indefinitely. It is, of course, perfectly clear that these cells do not produce other cells of a widely different character, but they are more nearly like embryonic cells, physiologically if not morphologically, than the cells from which they originally came. This would probably involve a sort of despecialization of the tissue cells with the resumption of an embryonic potency. The germ-plasm theory postulates a difference between the germ cells and the body cells of such a sort that the former are conceived to have the ability to live and develop indefinitely, while body cells have a limited life. The behavior of the cells in cancerous growths may do no more than show the ability of highly differentiated tissue cells, under unknown or poorly known conditions, to regain this power of repeated and indefinite growth; but this tends to break down the distinction between germ cells and tissue cells in this particular.

Loeb ('15), who has given much attention to the study of cancer cells, discusses the matter from that point of view. He concludes that the observations of fourteen years upon cancerous growths have established certain facts which are contrary to the view of the radical difference between germ cells and body cells. In those cases where it has been possible to detect and study the earliest indications of cancer in mice, he has been able to trace the transformation of the normal tissue cells into the abnormally proliferating tumor tissue, and is thus able to demonstrate the origin of the tumor from the tissue. He believes that germ cells and somatic cells are not so different, and possess no such differences in potency as is often claimed.

6. Summary and conclusions

The germ cells of Hydrozoa are differentiated, at a time just preceding sexual maturity, from different regions of the animal or colony, there being no one region or layer which characterizes the place of origin in this group. These germ cells probably arise in all cases from tissue cells; in some species such an origin is demonstrated, since an entire cell or half a divided body cell produces a single egg or sperm cell.

Budding in Hydra and hydroids involves a multiplication and growth of the cells and an evagination of all the body layers in the budding zone. The claim that latent germ cells are responsible for budding is not sustained by observations. Some medusae reproduce asexually by budding, and as a rule such buds are produced in a manner similar to that of hydroids, viz., by an evagination of both ectoderm and entoderm. In a few cases asexual buds of medusae arise from the ectoderm alone, but in no case does such a development come from a single cell. Buds may also come from the reproductive organs of medusae, but all investigators of this manner of budding agree upon the activity of ectoderm and entoderm cells of that region; such a process is not a development from germ cells. The different types of budding in Hydrozoa suggest an evolution of reproductive processes which may still be in progress. The phenomena of budding give evidence of a considerable degree of

plasticity in the cells of the body, a regressive change to an embryonic condition preceding the formation of the bud.

The germ-plasm theory invokes the aid of latent germ cells to account for regeneration, but there is no evidence of this in Hydrozoa. So many cases are recorded, in many groups of animals including vertebrates, of the de-differentiation of tissue cells and the formation of the regenerated structures from an indifferent or embryonic mass of cells, that it may be doubted whether regeneration is ever related to germ cells. When coelenterate tissues are ground up and the cells isolated, the latter coalesce to form masses capable of regenerating complete and normal individuals, but in all such masses the cells have become despecialized before the regenerative processes begin. The observations upon dissociated cells of hydroids show that germ cells, if present, degenerate and play no part in the ensuing regeneration, while the body cells, under the same stimulus, lose their specificity, become totipotent, and produce the variously specialized cells and differentiated structures of the normal individual.

Many animals of different phyla are known whose gonads are present at the breeding season and entirely unrecognizable at other times, in such cases the germ cells arise from the body cells of the appropriate region. Recent work upon mammals gives strong evidence of the degeneration of all germ cells formed during embryogenesis, the definitive germ cells only differentiating after birth from the germinal epithelium of the gonad.

Explanted tissues, grown in culture media outside the body, may undergo a de-differentiation and form cells more or less embryonic in character. Cancerous growths, originating from tissue cells, display a capacity for long-continued and apparently indefinite growth and division. Such facts are indicative of a less definite distinction between germ cells and body cells than has usually been maintained, and the possession of a considerable capacity in specialized cells to undergo a further differentiation, even in a new direction.

The investigations discussed in this section furnish a great body of facts utterly inconsistent with the theory of the con-

tinuity of the germ plasm. This seems to apply to many phyla, even to vertebrates, but is especially marked in the coelenterates. There are so many facts which contradict this theory that it may confidently be held not to apply in the coelenterates, at any rate.

IV. GROWTH OF EGG CELLS

1. Cytoplasmic growth

The growth of egg cells proceeds by several methods in animals; nourishment is obtained either without assistance from other cells, or else follicle cells, nurse cells, or other accessory structures assist in securing or preparing the nourishment for the egg. In none of the coelenterates is a follicle present nor are there nurse cells such as occur in insects. But there are two distinct methods of growth; one in which the food is obtained directly from the enteric cavity or from the adjoining cells, and the second in which neighboring cells are actually absorbed or engulfed. Often both methods may be employed. The cells which are absorbed have sometimes been called nurse cells, but they do not function in the way nurse cells do in other groups, since they are consumed instead of preparing food. Hydra, Tubularia, Pennaria, and Hybocodon are examples of those eggs which absorb neighboring cells for food, and Campanularia, Clava, Hydractinia, and Aurelia are examples of those which obtain food from the enteric cavity.

A different origin has been claimed for nurse cells and egg cells in those animals whose eggs are so nourished, the germ cells representing real reproductive cells while the nurse cells are held to be tissue cells. The Hydrozoa show no such distinction, for all the oogonia of any ovary are alike in origin and capable of becoming ova; the determination of which shall grow and which serve as food is largely a matter of chance. Even after growth has started, the surrounding cells are like them until degeneration phenomena become apparent in the cells undergoing absorption. One explanation for the initiation of growth is the presence of certain bodies in the cytoplasm. Schaxel ('10 a, '11 a) describes the growth of oocytes of Pelagia,

Aequorea, Forskalia, and Agalma as beginning only when chromatin passes from the nucleus into the cytoplasm. Jörgensen ('10) found similar bodies in the cytoplasm of Sycon sponges at the beginning of growth, Downing ('09) in oogonia of Hydra, and the author ('13 to '18) has noted an apparent correlation between the presence of such cytoplasmic granules and the initiation of the growth processes in the eggs of other Hydrozoa. None of these authors have expressed any thought of these cytoplasmic inclusions acting as indicators of germ-cell or tissue-cell origin, but Hegner ('14), who has collected data from many sources, explains them as germ-cell determinants.

After growth has once started, it continues rapidly, and reserve food is stored away for future use. The eggs of some Hydrozoa become filled with large yolk spheres, while in others the yolk is in fine particles so diffused through the cytoplasm as to be scarcely noticeable. There is a great deal of variation in the size attained by these eggs, as the figures and descriptions of the following section will show.

2. Nuclear growth

The detailed changes in the nucleus during growth have been described in the papers dealing with particular species; only certain more general relations are here discussed. As the eggs grow, their nuclei also increase, but not in the same ratio. Hertwig's suggestion of a constant ratio between nuclear and cytoplasmic volume is no more supported by the growing eggs of these coelenterates than it has been by other cells investigated by many workers. Jörgensen ('13) has made the claim of a definite relation between the relative size of the nucleus and the mode of nourishment of the egg, basing his claim upon observations of egg cells of a number of different animals. According to this author, eggs nourished by nurse cells or follicle cells, or by the absorption of adjoining ova and oocytes, have very small nuclei; eggs without special nourishing apparatus, but which absorb their food directly, possess relatively large nuclei. In the latter case, he believes, the nucleus of the egg is responsible for its

growth; in the former, the nuclei of the accessory cells govern the growth of the egg, and the nucleus of the egg is inactive till it enters upon the prophase of maturation mitoses.

A brief survey is sufficient to demonstrate a great variation in the relative size of nuclei in coelenterate eggs, and I have undertaken to test Jörgensen's suggestion. Figures 16 to 30 are the outlines of a number of eggs with their nuclei, accurately drawn to the same scale, all representing eggs at the end of the growth period before the prophase of maturation mitoses. Figure 15 is a similar representation of a starfish egg of the same stage, introduced for the sake of comparison. In the accompanying table these eggs are arranged in order, the one with the relatively largest nucleus heading the list. Since the nuclei are not always perfect spheres, and the eggs depart even more from a true spherical form, the figures given in the table for the diameters are averages of the greatest and least diameter of both structures. The measurements, in millimeters, were made from projected images; if each average is multiplied by 1000 and divided by 137 (the magnification of the projected images) the results will give the average diameters in microns. From these measurements was obtained the ratio $\frac{\text{diameter of egg}}{\text{diameter of nucleus}}$ indicated in the third column. The figures of this column, squared, give the ratio $\frac{\text{surface area of egg}}{\text{surface area of nucleus}}$, and the same figures, cubed, furnish the ratio $\frac{\text{volume of egg}}{\text{volume of nucleus}}$ (this computation is given in the last column of the table). The actual volumes are not important, the relative volumes being the thing desired. Some inaccuracies result from the computations based upon formulae for surface and volume of true spheres, but it is believed these are not great enough seriously to disturb the order given in the table. These figures also represent measurements and ratios for particular eggs, and are not of the nature of constants; there is variation in size of eggs of the same species, but this, again, is not of such magnitude as to modify the table greatly.

Table of measurements and computations of relative sizes of various coelenterate eggs, and their nuclei. Diameters, in millimeters, are made from projected images of the eggs and nuclei; these multiplied by 1000 and divided by 137 will give the diameters in microns. The diameters represent the average diameter of the egg and nucleus, since often these are not perfectly spherical

FIGURE	FORMS EXAMINED	AVERAGE DIAMETER NUCLEUS	AVERAGE DIAMETER EGG	DIAMETER EGG / DIAMETER NUCLEUS	VOLUME EGG / VOLUME NUCLEUS
15	Starfish.....	6.0	12.0	2.0	8.0
16	Nausithoë punctata....	8.0	20.0	2.5	15.625
17	Hydractinia echinata...	9.0	23.0	2.555	16.581
18	Pelagia noctiluca.....	11.0	30.0	2.7272	20.153
19	Obelia sp?.....	6.0	18.0	3.00	27.0
20	Aglantha digitalis.....	5.0	15.0	3.0	27.0
21	Campanularia flexuosa.	7.0	22.0	3.1428	30.957
22	Gonothyraea loveni....	4.0	14.0	3.5	42.875
23	Aurelia flavidula.....	5.0	18.0	3.6	52.656
24	Clava leptostyla.....	4.3	18.0	4.186	72.930
25	Corymorpha pendula...	6.0	39.5	6.583	284.848
26	Hydra sp?.....	7.0	47.0	6.714	302.6469
27	Eudendrium ramosum..	4.0	31.5	7.875	488.058
28	Pennaria tiarella.....	3.3	32.5	9.848	955.088
29	Hybocodon prolifer.....	4.0	58.5	14.625	3122.794
30	Tubularia crocea.....	3.0	54.5	18.166	5994.8435

Very obviously the table is divided into two parts, 16 to 24 represent eggs with relatively large nuclei, and 25 to 30 have distinctly smaller nuclei. Within each group there is a rather marked gradation, but between the groups a noticeable gap. The relation between the volumes of nuclei and cytoplasm may be expressed in another way. In the first lot the egg volume exceeds the nuclear volume by from 15 to 73 times, but the eggs of the second group are from 284 to nearly 6000 times the volumes of their nuclei. Each egg of the first lot obtains its nourishment from the enteric cavity, from which it is separated by a single layer of cells; the eggs of the second lot (except 27) absorb the surrounding oocytes and ova and appear to depend upon these almost entirely for their food supply. Eudendrium (27), in size of nucleus, belongs to the second series, but does not absorb oocytes; however, its gonophores are adapted to serve as nourishing organs, and the cells of these are later absorbed, so it may properly be placed in the second series instead of the first.

These fifteen coelenterate eggs support the claim of Jörgensen, or at any rate are consistent with his suggestion of the relation between the mode of nourishment of the egg and the size of the nucleus. Perhaps this agreement is incidental, for there are some objections to Jörgensen's views. His suggestion implies a passivity of the nucleus in eggs whose nourishment comes from absorbed ova. I believe, in these as in the others, there is an exchange of material between nucleus and cytoplasm of growing eggs, for there is evidence of the passage of chromatin into the cytoplasm of these eggs during growth. Nor does it seem probable that the nuclei of accessory cells could have anything to do in directing the growth processes, for in coelenterates these cells are absorbed and their nuclei may undergo a degeneration before absorption. All the facts sustain the belief that the nuclei of growing eggs are responsible for the direction of the functional activities of these cells. To this extent, at any rate, Jörgensen is probably incorrect in his interpretation. I think it quite probable that some relation may exist between the method of nourishment and the relative size of the nucleus, and the figures of the table may be an expression of this relation.

3. Cytoplasmic inclusions

In the cytoplasm of growing coelenterate eggs certain bodies occur as characteristic structures. These inclusions, described by the author ('13 to '18) as of nuclear origin, appear to be correlated with the growth processes, either furnishing the stimulus to growth or in some way determining the course and extent of growth. Similar bodies are present in germ cells of other animals at corresponding periods, but there is disagreement regarding their origin and function. Without doubt, some of the difference of opinion is due to the presence of cytoplasmic inclusions of different sorts, both as to origin and as to function. This is clearly established by the work of Cowdry ('16) and other recent writers.

In Campanularia the cytoplasmic bodies in the egg are formed from the dissolving nucleolus and passed through the nuclear wall into the cytoplasm, where they participate in the formation

of yolk. While growth begins before such bodies occur, the period of rapid growth is coincident with the passage of nuclear matter into the cytoplasm. The nucleolus is partly chromatic, and the bodies in the cytoplasm derived from the nucleolus also contain chromatin. Clava shows essentially the same phenomena, but the chromatin which passes into the cytoplasm appears earlier and comes from the nuclear reticulum, the nucleolus being a true plasmosome. After the chromatin enters the cytoplasm of Clava, growth begins. Growth begins in Aglantha shortly before nuclear substances enter the egg, or at least before definite cytoplasmic bodies can be recognized. In this egg it is not possible to determine the fate of the chromatin particles, except for their rapid solution within the cytoplasm, nor whether they have any close relation to cell metabolism. In the egg plasm of Hybocodon, chromatin granules appear before the growth of the oocyte begins; this migration of chromatin is abundant during early growth, but soon ceases, and the particles dissolve within the cytoplasm. Eudendrium shows similar inclusions in oocytes as growth begins, and they continue to form abundantly during practically the whole of the growth period. They are apparently of chromatic nature.

The interpretation of these cytoplasmic inclusions involves, chiefly, the consideration of their origin. Do such bodies arise, in the place where they first appear, out of materials of the cytoplasm, or do they represent nuclear substances in the cytoplasm? If the latter be the case, are the bodies composed of chromatin or of achromatic material? Bodies of cytoplasmic origin have commonly been called mitochondria, those believed to be chromatic in nature are sometimes referred to as chromidia. Tests seem to have demonstrated the reality and difference of these two classes of inclusions, for Cowdry ('16) believes, "we have ample evidence that the chromidial substance (Nissl substance) is a nucleoprotein containing iron . . . .., formed at least in part through the activity of the nucleus, and the mitochondria is a phospholipin albumin complex."

The granulations in the egg cells of the described coelenterates are certainly not mitochondria, though typical mitochondria

have been found in such cells, and no doubt are present in these. Their size, position, time and place of appearance, staining reactions, all seem to distinguish them as extruded nuclear material. They are present in young oocytes at the beginning of growth, and sometimes in later growth stages. They appear, in all cases, first, in the region of the nucleus, usually directly against the nuclear membrane; their appearance is often correlated with signs of activity within the nucleus and indications of currents in the cytoplasm; they stain like chromatin. Within the cytoplasm it is practically universal for them to lie within vacuoles, while other granules are commonly not so situated. In this latter respect they seem to produce a vacuolation or liquefaction of the surrounding cytoplasm in the same manner as Lillie ('02) described for chromatin particles which are free in the cytoplasm.

Jörgensen ('10) found a relation between egg growth and the presence of chromatin particles in sponge eggs; Schaxel, an emission of chromatin into the cytoplasm of coelenterates ('10 a, '11 a), Ascidia ('10 b), and echinoderms ('11 b); and the activation of the cytoplasm upon the entrance of the chromatin. Schaxel ('11 c) finds the mitochondria (chondriosomes) present in practically all cells at all times, while the extra nuclear chromatin (chromidia) occurs only at certain times, performs certain functions and disappears. He also recounts differences in appearance and staining reactions of the two sorts of bodies. Tsukaguchi ('14), using Altmann's technique upon Aurelia eggs, believes Schaxel to be in error, and considers all cytoplasmic granules as mitochondria. But the behavior of the bodies he investigated, especially their disappearance in later growth, is not like the usual behavior of the mitochondria.

Beckwith ('14) discusses the origin of the plasma structure of one of the hydroid eggs, and observes basically staining bodies, which she calls 'pseudochromatin-granules,' scattered through the cytoplasm. She also observed a second plasma granulation, "large drop-like masses which appear near the nuclear wall and which are also probably not chromatin;" these also are stained with nuclear dyes. Various stains were tried, and it was common

to find the nucleus and cytoplasmic granules staining alike, but some vital dyes gave a difference in staining reaction. If young eggs were digested in pepsin, the nucleus and the cytoplasmic granules were unaffected. Beckwith clearly points out the lack of precision in selective staining, but believes her evidence shows the non-chromatic character of the protoplasmic granules. "In all cases which seem to indicate the contrary conclusion (some staining and digestive tests and tests for proteid) the results can be interpreted in some other way." This author believes the contrary conclusions of Smallwood, Schaxel, and others are due to faulty technique. Differences in technique may undoubtedly account for difference in appearance, but it would appear rather improbable that these investigators, in addition to others not mentioned, all working independently and by different methods and arriving at similar conclusions, should not have worked out a reasonably satisfactory technique and should have been unable to distinguish between artifacts and real structures. It is permissible for Beckwith to differ in her interpretation of observed facts, but not to attack the methods of those who differ in this interpretation, with no more grounds than she offers. According to Beckwith herself, the evidence implies that these other authors were correct in interpretation; the weight of evidence of her own observations supports their contention of the chromatic character of the protoplasmic bodies under discussion, for she says, "the balance of the evidence . . . . indicates the non-chromatic nature of the granules in question." I do not believe the balance of her evidence outweighs the evidence in the other direction.

Jörgensen ('13) discounts his own earlier work on sponges, all of Schaxel's work, the work of Goldschmidt, Montgomery, and others, so far as they relate to questions like the present one. He believes undue weight has been placed upon staining reactions; it is necessary, in his opinion, to identify nucleic acid in plasm granules in order to show their chromatic origin. Pepsin digestion experiments convinced him of the presence of nucleic acid compounds in the cytoplasm of some eggs, and he admits the occasional migration of chromatin from nuclei, but he thinks this is

of no significance where it occurs. Jörgensen finds chromatin stains and mitochondrial stains and technique to be very uncertain, and neither of these, or any other staining method, is to be depended on, since they do not differentiate bodies of diverse origin and chemical composition.

An even stronger criticism of our staining methods and all microchemical tests is made by van Herwerden ('13). Our technique, she holds, is so primitive as to be useless in the identification of chromatin; evidence from stained, fixed preparations is not valid; action of weak or strong alkalis or acids does not give satisfactory results; digestion by pepsin and trypsin leads to no intelligible information; none of the usual tests are of any great service. This author uses nuclease as an enzyme in digestion experiments to test for chromatin (nucleic acid content) in the basic cytoplasmic granules of echinoderm eggs. Using ripe eggs, very simple experiments demonstrated the basophile granules of the cytoplasm to "consist of a nucleic acid compound." In younger oocytes, where chromidia had been described against the nuclear membrane, the nuclease experiments show the presence of nucleic acid compounds. Van Herwerden is somewhat doubtful as to the origin of these chromatin particles and hesitates to interpret it as a migration of chromatin from the nucleus. However, by observing living oocytes of Sphaerechinus, she could follow a movement of refractile granules to the nuclear membrane where they disappeared, and at the same time granules appeared in the cytoplasm close to the nuclear wall. Van Herwerden concludes that there is a possibility of the diffusion of nucleic acid compounds from the nucleus into the cytoplasm, but no direct proof of this. I suppose, in the very nature of the process, one could not expect to secure absolute proof of this passage, but van Herwerden seems to have obtained evidence which renders such diffusion highly probable. In all experiments with nuclease, the chromatin of the nucleus was affected in the same way (though to a much less degree) as the basophile granules of the cytoplasm. From the experiments and observations of van Herwerden there would appear to be ample warrant for the belief that nuclear material passes from the nucleus into

the cytoplasm of growing eggs; in other words the morphological conclusions appear to be supported by experimental results.

Outside the forms already mentioned, the insects are described as showing a passage of chromatin into the cytoplasm. Wassilieff ('07) finds the nebenkern of the cockroach spermatid has come from chromatin of the nucleus by a diffusion through the membrane. Hegner ('15), in the honey-bee and carpenter-ant, thinks the oocyte nuclei give off chromatin, which appears in the cytoplasm of fixed eggs as granules. In echinoderms Danchakoff ('16) finds basic granules, indications of cytoplasmic movements, and other conditions similar to those described by the author ('13) for Campanularia, but believes these mark the passage of basic material of the cytoplasm into the nucleus, where it becomes differentiated and helps to form chromosomes.

There are abundant records in the literature of the presence of basophile granules in the cytoplasm of eggs and other cells of animals. These have been observed and studied by cytologists, following their usual technique and have been interpreted in accordance with the morphological appearance; relatively few attempts having been made to check these by chemical or physiological tests. It would appear from some of the recent work that staining reactions are much less specific and selective than has been assumed; conclusions drawn from stained material, therefore, would have little significance and would be misleading, since morphological structures of a very diverse chemical composition and varied functions may stain alike. From this point of view, all interpretations based upon staining are of little value until they have been checked by appropriate chemical or physiological tests. I believe there is a large element of truth in these criticisms, and we have probably gone to an extreme in interpretations based upon purely morphological studies. For present purposes we are very fortunate to have had such a test of basophile granules of echinoderm eggs, with an application of these to the chromidial hypothesis of Goldschmidt and Schaxel. This hypothesis is not entirely substantiated by van Herwerden, and some of the 'chromidial apparatus' described for echinoderms is believed to be artificial. But the fundamental principle of the

theory is confirmed, viz., that basic granules in the cytoplasm contain nucleic acid components, which are similar to the nucleic acid compounds within the nucleus. Moreover, it appears quite probable that this cytoplasmic nucleic acid has come from the nucleus, van Herwerden having followed a nuclear emission in living echinoderm eggs. From this evidence we are warranted in believing that the passage of chromatic material (nucleic acid compounds) into the cytoplasm is a reality. According to the tests on echinoderms, it is the basophile granules near the nuclear wall in young oocytes which represent this material; probably the similarly placed granules in the coelenterate eggs are the same substance.

The determination of the functions of these bodies is not so simple, and there is a good deal of difference in interpretation. Hegner believes the chromatin bodies in egg cells are germ-cell determinants; Goldschmidt thinks they represent the chromatin which is responsible for all the vegetative functions of the germ cells; Schaxel looks upon them as regulating some of the cell functions, but not governing all vegetative activities; the author has held the view that they are related to yolk production, and possibly have an enzyme action in stimulating growth and synthesis of reserve food in eggs. Others view these bodies as of no significance in cell metabolism. If they play a single definite part in the cell metabolism, further work is necessary for a decision. My own impression would lead me to discard the view of a total absence of any significance.

V. CHROMOSOMES

The maturation phenomena, characteristic of germ cells, are exhibited by both male and female germ cells of coelenterates. In the egg cells polar bodies are formed by means of mitosis, and a reduced number of chromosomes remain in the egg. This reduction apparently takes place at the beginning of the growth of the oocyte, and evidence is not lacking of a conjugation of chromosomes. The coelenterates do not appear to offer material favorable for the determination of the method by which such conjugation is accomplished. Differences are noticeable in such

details as the form of spindle, distinctness of chromosomes, and the like, but the principles involved are those characteristic of similar phases in germ cells generally. In some instances conditions are found which have been interpreted as synizesis, in other cases such phases were not found. The coelenterates do not, therefore, add anything definite to the evidence concerning the normality of this process.

While the chromosomes appear to show a characteristic behavior, they are lacking in the variety of form and size which obtains in the chromosomes of some animals. In most coelenterates whose chromosomes have been studied, there is a similarity which renders it very difficult even to identify synaptic mates in maturation mitoses. Of the forms studied by the author only Aglantha had chromosomes which offered a reasonable opportunity for a study of details. Oogonial chromosomes did not, however, readily lend themselves to a grouping into homologous pairs. Some doubt was expressed as to whether these chromosomes behaved in quite the fashion believed to be characteristic and typical of maturation mitoses. The evidence is not sufficient to warrant any definite conclusions of a difference in the chromosome behavior of the coelenterates.

The question of the individuality and continuity of the chromosomes has been in mind during the study of the coelenterate germ cells. On one point the evidence is clear. During interkinesis there is no indication of the persistence of the chromosomes, the 'resting nucleus' is typically a single vesicle clearly without division into smaller vesicles. In certain forms chromosomal vesicles are produced after maturation or cleavage mitoses, but it is very common for two or more chromosomes to form a single vesicle. In any event, if the period of interkinesis is long, these vesicles unite into a single one. On the matter of the maintenance of chromosome individuality during interkinesis Wilson ('13) says:

> Some of the most careful recent cytological studies in this direction seem to show that such is not the case. Nevertheless these same studies, together with recent experimental evidence, give very strong ground for the conclusion that a definite relation of genetic continuity exists between the individual chromosomes of successive generations of cells.

The evidence obtained from coelenterate eggs would not permit one to dissent from this view. In the absence of contrary evidence in this group, the evidence from other groups would lead me to agree that there is no reason to believe the coelenterates differ in this regard.

Recently Robertson ('16), McClung ('17), and others have expressed a more radical view. Robertson believes the chromosomes are 'individually indentical' in succeeding generations and 'persist as entities' from one cell division to another. McClung is likewise convinced that each chromsome persists as a distinct structure; during interkinesis the chromosome may extend its boundaries and diffuse its substance, but each body retains just as precise a limit (though it is usually unrecognizable) during this period as it does during its stay in the usual form. This is a return to the older view of a distinct morphological individuality which Wilson and others have abandoned. McClung says of the chromosomes, "either they actually persist as discrete units of extremely variable form, or they are entirely lost as individual entities and are reconstituted by some extrinsic agency." It is quite unwarranted to state that extrinsic agencies are all that can explain a reintegration of chromosomes under these conditions. McClung gives us a very valuable critique of chromosome individuality, and, in his chief arguments, makes use of analogies between chromosomes and other organic behavior. The restitution of the normal form in regeneration and the production of a typical adult form by developing eggs are due to internal organization and not to 'some extrinsic agency.' On the same basis, the restitution of chromosome form is scarcely to be ascribed to external agencies, even if there have been a loss of identity in interkinesis. McClung contrasts organization with lack of organization in urging a persistent and continuous individuality, but organization does not involve preformation, as his discussion assumes.

In discussing chromosomal relationships Payne ('16) says: "It seems to me it is time we were realizing that evolution of chromosomes as morphological units, in chromosome numbers, and in chromosome behavior has been as diverse as it has been in

external morphological characters." Nor have we any reason to believe this evolution has ceased. It is quite conceivable that the chromosomes may be tending toward a persistence throughout the entire life of the cell in all its changes, and in some cases may now be distinguishable in interkinesis as well as in mitosis. But the evidence does not warrant a belief in such a continuity as Robertson and McClung postulate for all chromosomes of all organisms. The work of Hance ('17) furnishes him with no evidence of a persistence of individuality during interkinesis, and he can only subscribe to such a view by broadening the present concept. That is, he believes the chromatin particles may persist from generation to generation, but the bodies which they form do not persist. This view could hardly be tested, since we are without means of identifying or following particular chromatin particles at the present state of our technique. If such a belief could be confirmed, we should have a chromatin individuality hypothesis which would be without many of the objections of the present one.

So far as coelenterate chromosomes are concerned, there is nothing to disprove the view that the chromosomes of one generation are descended from the chromosomes of a previous generation. All the evidence obtainable, however, is quite inconsistent with the view of the persistence of chromosomes as distinct entities during interkinesis. A genetic continuity is very probable, a morphological continuity is highly improbable.

VI. SUMMARY AND CONCLUSIONS

In the Scyphozoa and Actinozoa all observations point to the entodermal origin of germ cells. The former widespread belief in the ectodermal origin of germ cells in Hydrozoa cannot be maintained, for literature records show a greater number of species whose germ cells arise in the entoderm than of those in which the ectoderm produces them. The germ cells of Hydrozoa may originate in either or both germ layers; the same individual may even produce germ cells from both ectoderm and entoderm. There is no characteristic place of germ-cell differentiation in this class.

The germ cells of some animals have been observed to form relatively early in ontogeny, but such is not the case in coelenterates. It has been claimed by some writers that in Hydra and a few hydroids germ cells are differentiated in the larvae. This claim has been refuted by later studies upon Hydra, and a new investigation of larval stages of hydroids furnishes no evidence of an early differentiation of germ cells in those forms. Furthermore, there are a number of the Hydrozoa whose germ cells have been observed to arise directly from differentiated body cells. This happens either by the transformation of an entire epithelial cell into a germ cell or by the division of a body cell and the transformation of one of these division products into a germ cell. In the latter case the sister cell persists as a functional tissue cell. In at least ten species of eight different genera the germ cells have been observed to form in this way. This positive evidence, together with the refutation of all contrary claims, points to a single conclusion, viz., in the Hydrozoa (probably also in all coelenterates) germ cells are not differentiated in early ontogeny, but only much later as the time of sexual maturity is at hand.

The theory of the continuity of the germ plasm postulates the formation of a somatic blastomere and a germinal blastomere at the first cleavage of the egg; in no animal is such a result known. As applied to hydroids, the theory originally admitted the origin of germ cells from histologically differentiated somatic cells, but invoked the aid of invisible and unrecognizable germ substance lying latent in such body cells. The production of a germ cell by the transformation of half a tissue cell, and the persistence of the other half as a tissue cell, is sufficient to disprove the claim of the presence of an invisible germ plasm in such tissue cells. This fact, together with the origin of germ cells only as sexual maturity approaches, indicates a lack of continuity of the germ plasm in the coelenterates.

As explained by the germ-plasm theory, budding is always due to the presence of latent germ cells. But budding in Hydra and hydroids involves the activity of all the layers of the budding zone; in Hydra it is possible that interstitial cells first

become active, but there is no evidence that germ cells are present. The budding phenomena of medusae resemble the same processes in hydroids, since, in most cases, both body layers evaginate to form the bud. In a few forms buds are produced from the gonads of the parent medusa, but even here this is not a germinal process, for the buds are formed from all layers of the animal; the germ cells of the gonad may participate in the process, but only by behaving as tissue cells. A few medusae form their buds from the ectoderm alone, and one investigator claims that the bud originates from a group of oocytes, though he admits this is a very unusual method, not applicable to most buds in coelenterates. In no case are buds known to arise from a single cell. While it may be possible, therefore, that budding is occasionally a germinal process in medusae, this is rare; as an alternative explanation, other investigators believe both germ cells and tissue cells are able to undergo regressive changes and become embryonic. The embryonic cells have the ability to form a new organism. The latter explanation would correlate various types of reproduction, both sexual and asexual, in coelenterates; would correlate fission and budding in coelenterates and other groups of animals, and would outline a possible evolution of reproductive processes in coelenterates. The germplasm theory, therefore, may be held not to apply to budding in coelenterates, for it is contradictory to most of the facts of this phenomenon.

Regeneration is also held to be dependent upon the presence of latent germ cells. There seems to be no direct evidence in favor of this view, and the great body of facts concerning regeneration in many phyla of animals contradict such an interpretation. Especially do the observations upon regeneration from isolated cells of hydroids disprove the germ-plasm theory. When hydroid tissues are broken up into isolated cells the latter undergo a despecialization and fuse to form syncytia. From these masses complete and normal hydranths are regenerated. When germ cells are present they are absorbed as food, and take no part in the regenerative processes. The behavior of the isolated cells has been followed with the microscope and

sections made of the regenerative plasmodia. All the facts point to the totipotency of the tissue cells under such stimulus.

In some animals of different phyla reproductive organs are present only during the breeding season, and at other periods no germ cells can be recognized. In such cases the germ cells must be differentiated from the tissues of the region which gives rise to the reproductive organs. There is no evidence of a continuity of the germ plasm in these animals. In the vertebrates, especially in mammals, recent observations point to the degeneration of all germ cells which are formed during foetal life; the definitive germ cells are differentiated from the germinal epithelium after birth.

Pieces of tissue removed from the body will grow in culture fluids, under certain conditions. In some cases the new growths from this explanted tissue are embryonic in character, due to a despecialization of the old differentiated tissues. Cancers, developed from tissues, are composed of cells more embryonic in character than those from which they arose. These cells may continue to live, grow, and divide indefinitely. Such observations indicate a less marked difference between body cells and germ cells, and a greater plasticity and a more varied potency in differentiated tissue cells, than has commonly been believed. Such a weakening of the line of demarkation between these two categories of cells tends also to weaken the germ-plasm theory.

So far as the coelenterates are concerned, the observations upon the time and method of germ-cell origin; upon budding of all types; upon regeneration of the usual sort, and regeneration from plasmodia formed by coalescence of isolated cells, all point in one direction, viz., that there is no germ plasm in the sense of Weismann. Furthermore, the origin of germ cells in some phyla other than Coelenterata, the despecialization of differentiated cells, and their behavior in tissue cultures and in normal development, and the continued growth and division of body cells in cancers, also present evidence contradicting the germ-plasm theory. There are so many facts, from such different sources and from so many phyla, which are inconsistent with

the theory, that it may be questioned whether the theory applies at all extensively to animals of any phylum.

As a rule, those coelenterate eggs which secure nourishment from the adjoining enteric cavity have large nuclei; and the ones which absorb oocytes or other cells possess relatively small nuclei. Whether this correlation be incidental, or whether it have a deeper significance, is not known.

Cytoplasmic granules which stain in nuclear dyes are a characteristic feature of coelenterate eggs. Typically, these appear in young oocytes about the time growth begins, and they may also form at other times during growth. From their initial position, close to the nuclear wall; from their staining reactions; from the behavior of other cytoplasmic and nuclear substances; the author has interpreted these granules as chromatin. Observations by other investigators, upon the eggs of other animals, have led them to conclude that chromatin does migrate into the cytoplasm. The criticism that the usual tests for chromatin are not specific is justified in large measure, but digestive experiments have demonstrated the presence of nucleic acid compounds in cytoplasmic granules, similar to those described for coelenterate eggs. Also van Herwerden has observed a migration of nuclear material into the cytoplasm of living oocytes of Echinoderms. Using these experiments to check the other observations, it seems probable that the cytoplasmic bodies described as chromatin do, in fact, represent this substance. There is considerable diversity of opinion as to the functions of these inclusions, and further work is necessary to determine this with certainty.

The chromosomes of most coelenterates do not lend themselves to a study of details of behavior to the degree possible in some animals. This is due, chiefly, to the lack of variety in form and size. It is not possible, therefore, to determine whether the chromosomes reappear in each generation in precisely the same form and size they had in earlier generations. During interkinesis the nucleus is a single vesicle with no subdivisions into smaller vesicles, and the chromatin is in the form of a continuous reticulum.

Since there is no evidence to disprove the view that chromosomes are genetically related, this may be accepted for the coelenterates. But all the evidence from this phylum is opposed to the view of a persistent morphological continuity and an individuality of the chromosomes retained during interkinesis.

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EXPLANATION OF PLATES

All figures of plates 1 and 3 were made by the aid of a camera lucida. These plates have been reduced in reproduction to three-quarters the original size; the magnifications given for each figure are the actual magnifications as reproduced. The figures of plate 2 are copied from the sources indicated.

PLATE 1

EXPLANATION OF FIGURES

1 to 4, Campanularia flexuosa, approximately × 1150; 5 to 8, Tubularia crocea, × 620.

1 Young planula with coelenteron present. Primitive ectoderm and entoderm present, but cells walls are to be detected in only a few places. Both ectoderm and entoderm are filled with yolk spheres.

2 Older planula with walls becoming more plainly marked. There are few interstitial cells in the ectoderm, but a number are present in the entoderm. The cell with the large nucleus may be like Wulfert's germ cell in Gonothyraea, but the nucleus is similar to that of other entoderm cells. Some of the interstitial cells are differentiating into gland cells, muscle cells, and the like. No germ cells are present.

3 Same planula as figure 2, showing only a portion of the entoderm. A group of typical interstitial cells is represented.

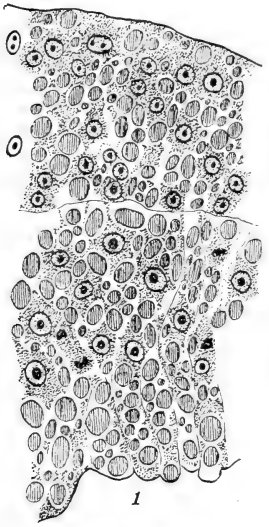
4 A still older planula with definitive ectoderm and entoderm. There are fewer entodermal interstitial cells than in earlier stages. The interstitial cells are undergoing differentiation, but no germ cells are present.

5 Section of egg about the end of cleavage. The germ layers have not been separated. The cell in division may be forming an ectodermal and an interstitial cell.

6 Embryo with a definite outer layer of cells and a solid central mass of cells. Two interstitial cells have been produced, one of which was formed by the division of a cell of the central mass.

7 A later embryo with cubical ectodermal cells, and groups of intersitial cells. One of the ectoderm cells is dividing to form an interstitial cell. None of these interstitial cells form germ cells at this time.

8 An embryo with coelenteron, about the period of the formation of tentacles and the production of an actinula. The ectoderm and entoderm cells are distinctly separated by a supporting lamella. Groups of interstitial cells are present and others are forming from the ectoderm. These interstitials form nematocysts and other structures, but not germ cells.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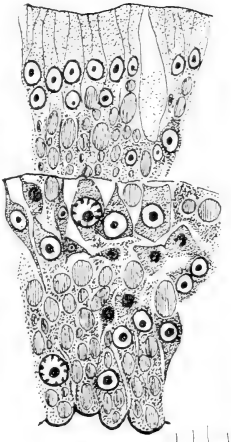
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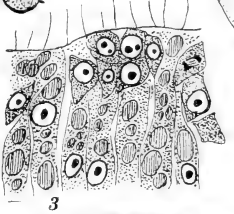
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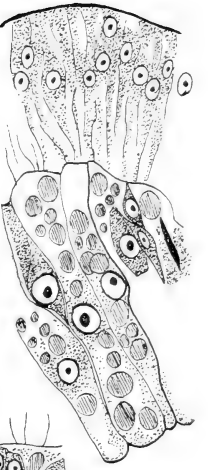
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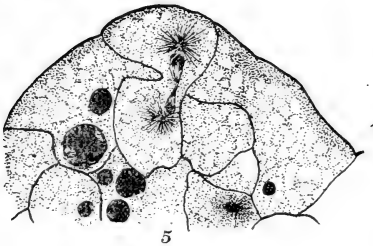
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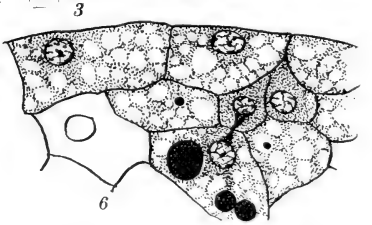
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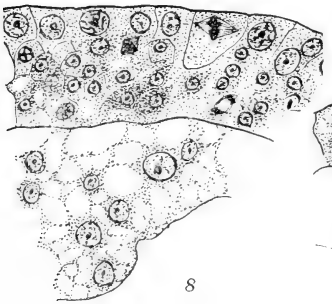
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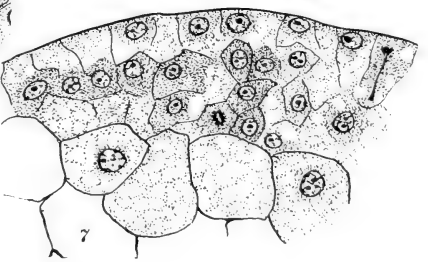
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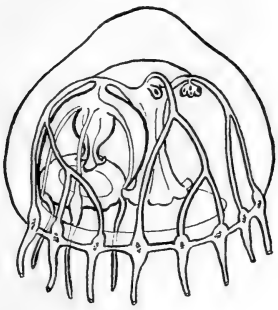
PLATE 2

EXPLANATION OF FIGURES

9 Proboscidactyla ornata. From Mayer, vol. 1, plate 21, fig. 5.
10 Sarsia gemmifera. After Chun, from Mayer, vol. 1, p. 63.
11 Niobia dendrotentaculata. From Mayer, vol. 1, p. 187, plate 19, fig. 2.
12 Eucheilota paradoxica. From Mayer, plate 37, fig. 3.
13 Sarsia codonophora. After Haeckel, from Mayer, vol. 1, p. 61.
14 Cytaeis atlantica. After Haeckel, from Mayer, vol. 1, p. 134.



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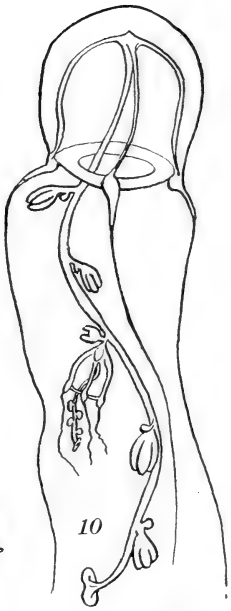

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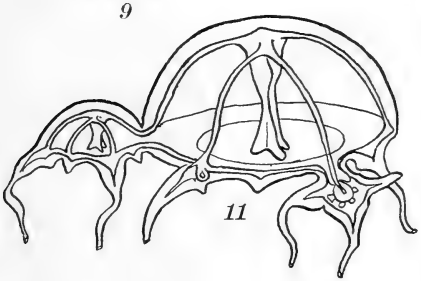

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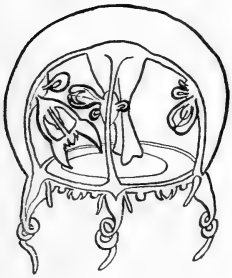

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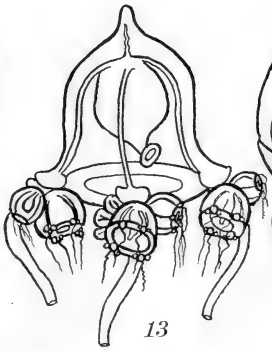

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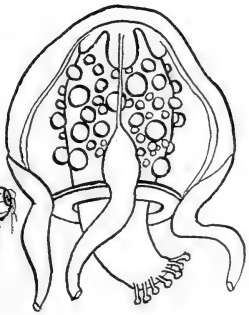

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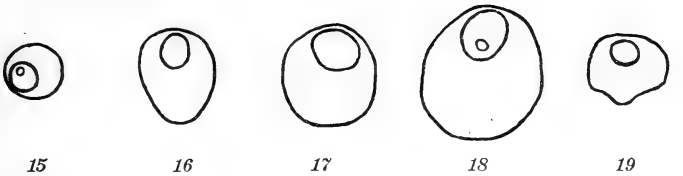
PLATE 3

EXPLANATION OF FIGURES

Drawing of eggs of coelenterates showing form and size of egg and nucleus at the end of the growth period. The eggs are arranged in the order of the relative volume of nucleus and egg. All drawn to the same scale, × 103.

15 Starfish egg, introduced for comparison of relative size and volume of egg and nucleus, with coelenterate eggs.
16 Nausithoë punctata.
17 Hydractinia echinata.
18 Pelagia noctiluca.
19 Obelia sp?
20 Aglantha digitalis.
21 Campanularia flexuosa.
22 Gonothyraea loveni.
23 Aurelia flavidula.
24 Clava leptostyla.
25 Corymorpha pendula.
26 Hydra sp?
27 Eudendrium ramosum.
28 Pennaria tiarella.
29 Hybocodon prolifer.
30 Tubularia crocea.



15 16 17 18 19

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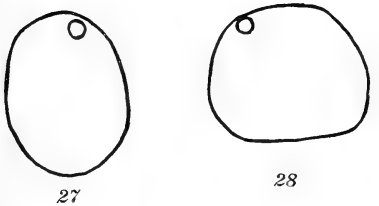

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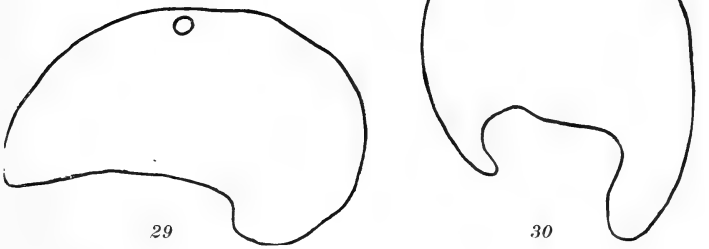

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Resumen por el autor, Carl L. Hubbs,
Universidad de Illinois.

Estudio comparado de los huesos que forman la serie opercular en los peces.

Aunque la estructura y disposición de los huesos que forman la serie opercular de los peces han sido descritas en ciertos grupos por muchos anatómicos, ninguno parece haber consolidado todavía las pruebas obtenidas, mediante un estudia comparado. Después de examinar la estructura de estos huesos en una extensa serie de peces, el autor ha llegado a la conclusión de que son de tipo diferente en los Malacopterigios más primitivos, por una parte, y en los Acantopterigios más especializados y sus parientes más próximos, por otra. Los Isospondyli, el grupo más primitivo de peces teleósteos (tal como se definen generalmente), tienen places operculares y radios branquióstegos bastante semejantes a los de Amiatus, que bajo este aspecto como en tantos otros, constituye la forma de transición que llena el hueco existente entre los Ganoideos y Teleósteos. En los otros grupos principales de peces con radios blandos (Ostariophysi, Stomiatoidea, Apodes, Heteromi, Lyopomi, Synentognathi, Haplomi, Iniomi) los opérculos y los radios branquióstegos son de tipos derivables aparentemente de los presentes en los Isospondyli. Hay sin embargo una extensa variación en la forma y disposición de estos huesos, en armonía con su posición generalizada. Los grupos más especializados de los teleósteos, por otra parte, (los Microcyprini, Labyrinthici, Hemibranchii, Symbranchii, Opisthomi, Salmopercae y el vasto conjunto comprendido en los Percoidea o relacionados con este grupo) retienen constantemente una disposición peculiar fija en los radios branquióstegos.

Translation by José F. Nonidez
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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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A COMPARATIVE STUDY OF THE BONES FORMING THE OPERCULAR SERIES OF FISHES

CARL L. HUBBS

Although the structure and arrangement of the bones comprising the opercular series of certain species or groups of fishes have been described by many anatomists, no one seems to have consolidated the evidence in a comparative study. After examining their structure in a wide range of fishes, the writer has concluded that they are of a different type in the more primitive malacopterygians, on the one hand, and the specialized acanthopterygians and their relatives, on the other hand. The Isospondyli, the most primitive of the teleosts (as usually defined), have opercular plates and branchiostegal rays similar to those of Amia, which, in this respect as in many others, bridges the gap between ganoids and teleosts. In the other chief groups of soft-rayed fishes—Ostariophysi, Stomiatoidea, Apodes, Heteromi, Lyopomi, Synentognathi, Haplomi, Iniomi—the opercula and branchiostegals are of types apparently derivable from that of the Isospondyli. There is a wide variation in the form and arrangement of these bones in these groups, however, as one might expect from their generalized nature. In the higher groups of teleosts, on the other hand—the Microcyprini, Labyrinthici, Hemibranchii, Symbranchii, Opisthomi, Salmopercae, and in that vast assemblage of modern types comprising in the Percoidea, or clustering about that group—there is maintained a peculiarly constant arrangement of the branchiostegal rays. From a taxonomic standpoint, the results of this study are most significant in confirming many of the recent refinements, particularly those suggested by Mr. C. Tate Regan, in the classification of the Teleostei.

The primitive structure of the membrane bones which support the outer wall of the branchial cavity, and thus protect the

branchiae, is retained in certain extinct groups of ganoids, such as the Palaeoniscidae. Their structure in this family has been described by Traquair and others. On each side is a curved series of homologous, suturally conjoined plates, of which the upper two or three are dilated to form the opercular bones, while the lower anterior ones, narrower and less modified, comprise the branchiostegal rays (or branchiostegals); the lowest or most anterior pair, enlarged to form the plates which may be termed the branchigulars (or branchigular plates), are suturally united posteriorly with one another, and anteriorly with the median gular plate (or intergular), which extends forward between the mandibular rami to their symphysis.

In Amia calva the bones of the opercular series are modified in several respects. Each of the upper five plates is imbricate over the one next below. The gular plate largely covers the most anterior branchiostegal rays of the left side, which in turn overlap those of the right side—a feature due to the definite asymmetric folding of the branchial membranes, an asymmetry which is more or less definitely retained throughout the entire teleost series. The uppermost and largest bone of the opercular series in Amia is the operculum, a large subquadrate plate, incurved and thickened along its dorsal and anterior edges, and provided inside the anterior edge with an oval socket, into which a peg-like condyle of the hyomandibular fits, allowing considerable lateral movement of the operculum. The suboperculum, the next lower element of the series, is broadly incised by a downward extending arm of the operculum; its dorsal edge slips just under the lower margin of the operculum, while its lower edge is closely joined by membrane with the triangular interoperculum. The anterior edges of these three plates fit into a posterior groove of the preoperculum, which is not regarded as a member of the opercular series. A free dermal fold connecting the opercular membrane with the mandible, and extending along the lower edge of the sub- and interoperculum, may be termed the subopercular fold. The fourth bone of the series, fitting between the sub- and interoperculum, but widely exposed, being nearly one-third as wide as long, extends downward and forward, lying free in a conspicuous fold, continuous with the lower edge of the

mandible. This plate, bearing as much resemblance to the opercula as to the branchiostegals, may be named the branchioperculum; its fold, the branchiopercular fold. The remaining ten plates of the series are attached to the ceratohyal element of the hyoid arch, near the lower edge of its outer face. Except for its pointed, rather than truncate anterior edge, the uppermost of the ten is similar in form to the branchioperculum, which overlies its dorsal edge. Both this plate and the next, which is similar, though only about half as wide, are imbricate on the one next below, having free folds along their lower edges. The lower anterior seven branchiostegals form a continuous even surface, each, except the uppermost, fitting tightly into a groove along the lower outer face of the one next above; they increase in width anteroventrally. The most anterior branchiostegal, which is wider on the left or outer than on the right side, may be homologous with the branchigular plate of the Palaeoniscidae, or may represent two or three fused branchiostegals. The current allocation of Amia in a position intermediate between the typical ganoids and the teleosts is confirmed by the study of its opercular and branchiostegal plates.

The Isospondyli, comprising the oldest and most primitive[1] of the teleosts, retain certain generalized features of the opercular series. Thus, in Elops an intergular plate is developed, and in Albula, although the plate itself is lacking, the intergular fold remains. The branchiostegals of the typical Isospondyli (at least the upper ones), persist as thin wide plates. The uppermost and widest ray (which may be termed the branchioperculum, as it seems to be homologous with the plate in Amia to which that name is here applied) is attached closely to the inner margin of the sub- and interoperculum; not having become concealed under these bones, it remains visible from the side. The whole series, in fact, remaining scarcely at all folded together after the fashion of a fan, is visible from below,[2] though the branchial membranes

[1] Excepting of course Lepidosteus and Amia, if these be included in the Teleostei.

[2] In the clupeoid fishes the expanded preoperculum covers the larger portion of the middle rays, and all of the rays are mostly concealed in Chirocentrus dorab.

are separate (as they usually are). The plates of the opercular series in the isospondylous fishes differ from those of Amia in the following respects: the reduction of the suboperculum, so that the interoperculum and operculum are in contact anteriorly;[3] the proximal (or anterior) attachment of branchioperculm and branchiopercular fold to the hyoid arch; the more complete imbrication of all the rays; the attachment of branchiostegals to the epihyal as well as to the ceratohyal; the frequent reduction of the rays below the main hyoid suture to rather slender rods, and the occasional attachment of these reduced rays to the edge of the ceratohyal, rather than to its outer face. These last two features are apparently caused by the strong development of the musculus geniohyoideus of the lower jaw, which is attached to the hyoid arch near the suture separating the ceratohyal from the epihyal. The number of the larger and flatter rays attached to the outer surface of the epihyal (the lowermost sometimes on the suture) varies widely in the Isospondyli and related orders; the writer has counted one in Bathylagus pacificus; two in Pterothrissus gissu, Hiodon tergisus, Osmerus thaleichthys, Osmerus attenuatus, Arius gagora, and Amiurus nebulosus; three in Amphiodon alveoides Ethmidium maculatum,[4] Alepocephalus agassizii, Coilia ectenes, and Hypomesus olidus; either three or four in Albula vulpes; four in Chirocentrus dorab, Salvelinus fontinalis, Osmerus mordax, Mallotus villosus, Plagyodus ferox, Lestidiops sphyraenopsis, Bathysaurus ferox, Chlorophthalmus chalybeius, and Neoscopelus macrolepidotus; five in Etrumeus micropus, Felichthys felis, and Dallia pectoralis; six in Oncorhynchus nerka, Saurida gracilis, and Bathypterois pectoralis; either six or seven in Trachinocephalus myops; seven in Esox lucius, Aulopus japonicus, and Synodus intermedius; eight in Esox americanus and Synodus

[3] According to Woodward's restoration of the primitive extinct genera Leptolepis and Holcolepis, these genera bridge the gap between Amia and Elops in the character of the opercula. These bones apparently show no significant variation among living teleosts, though certain ones are reduced or increased in size in certain genera; the interoperculum is especially liable to variation, being occasionally absent.

[4] All but the lowermost of the six on the ceratohyal are of similar shape to those of the epihyal in Ethmidium maculatum.

lucioceps; nine in Elops affinis and Harpodon microchir; ten in Megalops atlanticus. The total number of branchiostegals is three in the Cyprinidae and others, twenty-four to thirty-six in the several species of Elops. Many other figures might be added, but these are enough to illustrate clearly the inconstancy of the number of branchiostegal rays in the generalized malacopterygian fishes.

In the groups of soft-rayed fishes other than the Isospondyli, the branchiostegals are variable in form and attachment, but they show many points of similarity to those of the Isospondyli. In the Ostariophysi the number of rays varies widely, but the uppermost, at least, remains like that of the isospondylous fishes. To take several examples from the Nematognathi, there are six branchiostegals in Arius gagora, seven in Pseudeutropius garna and Saccobranchus fossilis, nine in Ictalurus punctatus, Amiurus nebulosus and Shilbe mystus, eleven in Macrones aor. The characins have only three to five branchiostegals, the cyprinids, constantly three. This low number of branchiostegals in certain malacopterygian fishes is usually correlated with the broad union of the branchial membranes and with a fresh-water habitat. Similarly, there are only three branchiostegals in Haplochiton, Phractolaemus, Kneria, and Cromeria, and but four in the Gonorhynchidae, Chanidae, and Salangidae (in all of these, excepting Gonorhynchus,[5] the uppermost ray remains visible below the margins of the opercles). In the Mormyridae and Notopteridae the branchiostegals are modified in various ways, as Doctor Ridewood ('04, pp. 191–195, 199, 205) has demonstrated; in Notopterus there are six to nine branchiostegals, in Xenomystus but three, according to Boulenger.

The stomiatoid fishes, formerly confused with the Iniomi, have the branchiostegals short, slender, little curved, evenly spaced, not folded together, attached to the external surface of the hyoid arch near its ventral edge (each opposite a photophore), and largely covered by the opercula. In the Apodes (eels), Heteromi

[5] In Gonorhynchus there are four branchiostegals, attached beneath the opercles on the outer face of the club-shaped end of the hyoid arch, all above the suture between the ceratohyal and epihyal.

and Lyopomi, the rays are also all slender, usually numerous and long, and frequently curved upward posteriorly about the free margin of the opercular bones. The branchiostegals of the Synentognathi (Belonidae, Scombresocidae, Hemirhamphidae, Exocoetidae) are wholly similar to those of the typical Isospondyli; they are rather numerous (ten in Euleptorhamphus), but not constant in number, flat, imbricate plates; the uppermost skirting the lower margins of the opercula, and all with their lower edges exposed. The characters of the branchiostegal rays of the Synentognathi strongly confirm Regan's view that the resemblance between these fishes and the Percesoces is purely fictitious: the group should be placed among the typical soft-rayed fishes. In the Haplomi (Esox, Umbra, and Dallia), but not in the poecilioid fishes which have been confused with them, the branchiostegals are like those of the Isospondyli. In the Iniomi (the Synodont fishes and their allies) the branchiostegals vary greatly in number (from six to twenty, four to eight attached to the suture between ceratohyal and epihyal, two to twelve below the suture); in Plagyodus the uppermost ray, as in the Isospondyli, is not wholly concealed, but in most of the genera several of the upper rays are covered by the opercula; when the rays are numerous several of the upper ones are closely approximated basally.

The group of the ribbon fishes (Taeniosomi) has been accorded very different positions among fishes, the current tendency being to place it much lower in the series than formerly, a disposition of the group which is doubtfully confirmed by the arrangement of the branchiostegal rays. In Regalecus, according to Parker's figure ('86), there are six slender, saber-shaped branchiostegals, all attached to the outer face of the hyoid arch near its lower margin; the uppermost, the only one attached to the epihyal, curving around the lower margin of the interoperculum. In the still more extremely aberrant genus Stylephorus, as described by Starks ('08), the five rays are inclined upward from their origin near the upper edge of the ceratohyal, as in no other known fish. In Trachypterus arcticus, as described by Meek ('90), the branchiostegal rays differ to no considerable degree from those of Regalecus. As in that genus, they are six in number; the uppermost

borders the lower margin of the interoperculum; all seem to arise from the outer face of the hyoid arch, but the anterior two are somewhat separated from the upper posterior four, which, unlike those of Regalecus, are largely covered by the expanded preoperculum. In Trachypterus rex-salmonorum the branchiostegals are concealed by the interoperculum as well; and the lower two rays, considerably separated from the upper four, are attached to the outer side of a ligament which extends as a chord across the concave anteroventral margin of the hyoid arch.

The Ammodytoidea are another group which has been placed by some ichthyologists among the higher teleosts, by others among the lower. The branchiostegals in Ammodytes personatus resemble those of the Acanthopteri in most of their characters: they are six in number, and are folded up behind the opercula; the upper four arise from both the cerato- and epihyal behind a prominent angle of the arch. The lower two rays, however, arise from the outer surface of the arch, and are closely approximated to the upper four.

The Microcyprini (Poeciliidae and Amblyopsidae) were long confused with the Haplomi, but have recently been shown to have a more advanced organization. The structure of the branchiostegal rays in the two groups confirms this view: those of the Haplomi are quite like those of the Isospondyli, whereas those of the Microcyprini are similar to those of the Acanthopteri. In the Poeciliidae there are six, or fewer, branchiostegals, which are folded up behind the operculum and above its lower margin. The upper four saber-shaped rays are attached to the outer surface of both the ceratohyal and epihyal, postero-superior to the prominent angle of the hyoid arch; the lower rays arise from the inner face of the ceratohyal. In examples of the Ophicephalidae and Anabantidae at hand (representing the order Labyrinthici), there are four plus two branchiostegal rays, arranged as in the Microcyprini and Acanthopteri.

Many of the aberrant fishes referred to the order Hemibranchii have the branchiostegals reduced in number, but in Fistularia there are four plus one rays, arranged as in typical Acanthopteri. "Most Lophobranchs have two branchiostegals, but Nerophis has

only one which distally bifurcates" (Jungersen, '10). In other respects also the hyoid apparatus of the Lophobranchii is reduced, probably from a condition like that of Fistularia.

The Symbranchia were long considered a group of true eels, but lately have been accorded a distinctly higher position. The character of the branchiostegals are in harmony with the latter view. In Monopterus javanensis, the rather narrow hyoid arch bears two groups of slender branchiostegals: an upper cluster of four and a lower inner pair, widely separated from the others. An essentially similar condition is developed in Symbranchus marmoratus, but in this species the two groups of branchiostegals are less widely separated.

The curious Opisthomi (Mastacembelidae) of southern Asia and Africa have been variously located in the teleost series; lately Boulenger and Regan agree in placing them among the higher teleosts, considering them as bearing a relation toward the spiny-rayed fishes analogous to that which the Apodes bear toward the soft-rayed group. This view is sustained by the branchiostegals in Mastacembelus pancelas. From the outer surface near the lower edge of the ceratohyal and epihyal, along the upper widened portion of the hyoid arch, four rays arise in close proximity; they are curved upward posteriorly, as in some of the Apodes, between the operculum and the branchial aperture; on the inner surface of the arch, near the concave anterior ventral margin, the two lower anterior rays are inserted.

The Salmopercae, long considered as intermediate between the soft-rayed and spiny-rayed fishes, have six branchiostegals, arranged exactly as in the Acanthopteri. Both of the species usually referred to this group, Percopsis omisco-maycus and Columbia transmontana, have been examined. Aphredoderus sayanus, referred by Regan to the same group, has branchiostegals in all essential respects similar to those of Percopsis and the following groups.

A definite fixed type of branchiostegal structure has been retained, almost without deviation, throughout the great groups of spiny-rayed fishes which flourish so abundantly in the modern seas, and with peculiar constancy in the numerous highly special-

ized offshoots of the typical Acanthopteri. In fact, it seems safe to assert that none other of the known characters which separate this series from the lower teleosts has been more conservatively maintained throughout the entire group. This statement may be emphasized by the naming of a few of the more aberrant types which differ in some notable way—primitive, specialized, or degenerate—from the group as a whole, yet which agree with one another and with the more typical members of the series in the essential characters of their branchiostegal apparatus: Atherina, Stephanoberyx, Plectrypops, Cepola, Psettus, Toxotes, Monacanthus, Lactophrys, Tetraodon, Diodon, Agonus, Cyclopterus, Cephalacanthus, Echeneis, Solea, Callionymus, Xiphidion, Scytalina, Gobiesox, Coryphaenoides, Antennarius, Ogcocephalus, etc. Broad union of the branchial membranes or their complete separation, membranous or fleshy character of the branchiostegal membranes, narrow lateral restriction or wide development of the branchial aperture, and countless other modifications of these higher teleosts occur—modifications affecting almost every part and structure of the body, as well as the branchial membranes—nevertheless, the essential characters of the branchiostegals remain unaltered.[6]

The characteristically stout hyoid arch is strongly angulated[7] at some distance below and before the (typically) dentate suture between the ceratohyal and the epihyal, the angle forming the hinder border of a concavity in which the musculus geniohyoideus is attached. The strong development of this muscle not only modifies the form of the hyoid arch, but also modifies the structure and attachment of the branchiostegal rays, as it also does, usually

[6] Certain of the individual rays may become reduced or specialized: for example, in Tetraodon the uppermost ray basally is an unossified ligament, while the lowest ray (as in Diodon) is greatly expanded; in Holotrachys the third to the seventh branchiostegals are strongly armed externally by rows of spinules; in Polymixia the lower three rays are modified, according to Starks, into a skeletal support for the barbel.

[7] The hyoid arch is also angulated, but in not quite the same way, in a few of the soft-rayed types, notably in Brevoortia, Dorosoma, Notopterus, and Gonorhynchus. In most of the lower teleosts the hyoid arch is a thin plate, and the suture between the epihyal and the ceratohyal is straight and often margined with cartilage.

to a lesser degree and without constancy, in the lower teleosts. The upper four saber-shaped branchiostegals are always attached to the outer surface of both epihyal and ceratohyal, at and above the angle of the arch, and are folded together like a fan above and behind the opercular margins (except in those cases in which the branchiostegal membranes are drawn taut by their broad union ventrally). Below (and before) the angle of the arch, to its edge or inner surface, usually two or three shorter and slenderer rays are attached; these may be reduced to one, or, very rarely, to none, and are increased, in certain berycoids and blennioids to four, but never to a higher number. Thus, the branchiostegals of the Acanthopteri and related groups are usually four plus two or four plus three in number, rarely four plus one or four plus four, and very rarely four plus nought or even three plus nought.[8]

In formulating the generalizations outlined in the preceding paragraph, one to many species of each of the families of higher teleosts, named in the following list, were examined. The variations in the characters of the branchiostegals rays were found to be so slight that for present purposes detailed descriptions are unnecessary.

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[8] A number recorded only for certain cirrhitiform percoids, so far as the writer has determined.

A list of the families of the spiny-rayed fishes (Acanthopteri) and their derivatives examined[9]

Atherinidae	Cepolidae	Gobiidae[12]
Mugilidae	Cirrhitidae	Echeneidae
Sphyraenidae	Embiotocidae	Bothidae
Stephanoberycidae	Cichlidae	Pleuronectidae
Polynemiidae	Pomacentridae	Soleidae[13]
Zeidae	Labridae	Trachinidae
Berycidae	Scaridae	Nototheniidae
Holocentridae	Scorpididae	Pteropsaridae
Polymixiidae	Toxotidae	Bathymasteridae
Scombridae	Ephippidae	Uranoscopidae
Carangidae	Ilarchidae	Callionymidae
Coryphaenidae	Acanthuridae	Clinidae
Leiognathidae	Siganidae	Blenniidae
Centrarchidae	Balistidae	Stichaeidae
Percidae	Monacanthidae	Xiphidiidae
Apogonidae	Ostraciidae	Lumpenidae
Centropomidae	Tetraodontidae	Pholididae[14]
Serranidae	Diodontidae	Anarhichadidae
Lobotidae	Scorpaenidae	Scytalinidae
Priacanthidae	Anaplopomatidae	Zoarcidae
Lutianidae	Hexagrammidae	Ophidiidae
Haemulidae	Platycephalidae	Brotulidae
Sparidae	Cottidae	Batrachoididae
Gerridae	Agonidae	Gobiesocidae
Kyphosidae	Cyclopteridae	Gadidae
Mullidae[10]	Cyglogasteridae	Coryphaenoididae
Sciaenidae	Triglidae	Lophiidae
Champsodontidae	Cephalacanthidae	Antennariidae
Malacanthidae	Eleotridae[11]	Ogcocephalidae

[9] The sequence of families adopted by Doctor Jordan in his Guide to the Study of Fishes ('05) is here followed.

[10] Branchiostegals four plus naught in the species examined.

[11] Branchiostegals four plus two or four plus three in all the genera examined, Eviota excepted.

[12] Branchiostegals four plus one in the numerous genera studied.

[13] The branchiostegals of all the flat fishes examined are of a very similar type.

[14] The writer follows Regan in the classification of the blennioid fishes.

Resumen por J. S. Kingsley, por el autor, Georgo Orihay Shinji.

Embriología de los Cóccidos, con especial mención de la formación del ovario, origen y diferenciación de las células germinales, capas germinales, rudimentos del intestino medio y los organismos simbióticos intracelulares.

Los óvulos, células nutridoras y las células del epitelio folicular se originan a expensas de las células germinales primordiales. Cuando el huevo entra en el oviducto posee las cubiertas ordinarias y una colonia de simbiotos. El blastodermo se forma por la emigración de una parte de las células de segmentación hacia la superficie, mientras que el resto de ellas forma las llamadas células vitelinas. La formación de la placa ventral es de tipo invaginado, solamente la pared dorsal del tubo asi formado es embrionaria, constituyendo el resto la capa amniótica. Los apéndices comienzan a esbozarse en el siguiente órden: labio, maxilas, patas torácicas, mandíbulas, antenas y labro. Existe una verdadera gástrula. Los neuroblastos son teloblasticos y dan lugar, por mitosis, a ganglioblastos. Existe una revolución del embrión. Un poco antes de entrar en el oviducto, el huevo es invadido por una colonia de organismos globulares o en forma de bastón, que se rodean de células germinales cuando se diferencian estas últimas.

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EMBRYOLOGY OF COCCIDS, WITH ESPECIAL REFERENCE TO THE FORMATION OF THE OVARY, ORIGIN AND DIFFERENTIATION OF THE GERM CELLS, GERM LAYERS, RUDIMENTS OF THE MIDGUT, AND THE INTRACELLULAR SYMBIOTIC ORGANISMS[1]

GEORGO ORIHAY SHINJI

TWENTY PLATES (ONE HUNDRED THIRTY-THREE FIGURES)

CONTENTS

1. INTRODUCTION

Historical

On account of their economic importance, scale insects have been the object of extensive observations by several investigators. The literature pertaining to these insects is, however, limited in its scope, being mostly concerned with the external morphology, general accounts of the life history, habits, and methods of control. The accounts of the formation of the egg and subsequent history of the development of coccids are mostly

[1]A thesis submitted to the faculty of the Graduate School of the University of Missouri for the degree of Doctor of Philosophy.

fragmentary. One of the earliest works of this kind was by Leydig ('54), who described and figured the general appearance of the ovaries of Lecanium (Coccus) hesperidum and its eggs with three nurse cells and an egg cell. Although he did not actually describe the process of differentiation of the ovarian elements, he claimed that nurse cells, egg cell, and the epithelial cells must have arisen from undifferentiated germ glands. He also described the formation of the embryo from the egg by multiplication of the single egg cell. The statement that this form is really viviparous was made in this article. He also pointed out the presence of numerous pseudonavicellae in this insect. These organisms, according to him, migrate into the egg at its posterior end and multiply rapidly by budding.

Leuckart ('58) has also studied the ovarian structure of Lecanium hesperidum and found that three nurse cells and an egg cell developed from epithelial cells. Therefore he maintained, as his predecessor did, that the nurse cells and the egg cell are the modifications of the epithelial cells.

Lubbock ('59) also came to the same conclusion, namely, the nurse cells, the egg cell, and the epithelial cells are all originally undifferentiated cells of the germ rudiment.

The most complete account of the development of coccids was, however, presented by Mecznikow ('66). His work on coccids is not so complete as was that for Aphids, Corixa, and Cecidomia. Nevertheless, it covers the development of the Aspidiotus nerii from a single egg-cell stage to the time of hatching. He described and figured a differentiated egg with its germinal vesicle. The appearance of the 'Wulst' prior to the formation of the blastoderm and the invagination of the blastoderm near the posterior pole of the egg were also mentioned. One layer of the invaginated 'Keimhügel' degenerated to form the amnion while the other developed into the embryo proper. However, he failed to observe the phenomenon of revolution of the embryo. The entire alimentary canal, he thought might be formed by further elongation of both stomodeum and proctodeum. He described and figured an early appearance of the germ cells and of the pseudovitellus.

Brandt ('89), who likewise studied Lecanium hesperidum and Aspidiotus nerii, with special reference to the embryonic coverings, stated that the embryo of Aspidiotus nerii was bent, as Mecznikow had already described, with its caudal part over the oral portion. He further observed the process of the revolution of the embryo following the rupture of the amnion. In Aspidiotus, he observed, as did also Mecznikow, that the ventral plate of the embryo was found lying closely on the amniotic covering, even before the revolution, so that the yolk was rapidly removed from this region.

Then followed the work of Putnam ('78) on the cottony maple scale, Pulvinaria innumerabilis. This writer evidently thought the ovarian eggs were in some manner, unknown to him, attached to the body cavity by their free or anterior end, where the differentiation first takes place. Besides this mistake, his figures are too vague to show anything very definite. However, this much was sure, that the eggs of the cottony maple scale developed within the body of the female. He detected the presence of the pseudonavicellae in the female before mating and also in the older eggs. The method of infection or the possible migration of these bodies into the eggs was not studied. However, he suggested that these bodies, having higher specific gravity than water, may represent the metamorphosed state of spermatozoa, and that their presence in the egg may be comparable to the phenomenon of fertilization.

Witaczil's ('86) work on the anatomy of the coccids contains an account of the differentiation of the nurse cells and of the egg cell from undifferentiated epithelial cells in the ovaries of Leucaspis pini. Although not figured, there is an account of the presence of the so-called nutritive string between the nurse chamber and the egg chamber. He did not find the pseudovitellus in the eggs of several species, but expressed the view that the pseudovitellus of Mecznikow may represent a mass of yolk granules.

The investigations above mentioned were entirely made upon fresh material or at least upon material prepared in toto. The eggs were usually studied in water to which acetic acid and sugar were added, or in somewhat similar solutions. The only paper,

the result of the study of the sectioned material, fixed and stained in accordance with modern microscopic technique is the contribution by Emeis ('15). The sole purpose of his article was to present the history of the three ovarian elements, namely, the nurse cells, the egg cell, and the epithelial cells. He did not, however, show whether the epithelial cells, from which the egg and the nurse cells develop, come from the primordial germ cells or from the original mesoderm. He was also not sure whether or not a quantitative or qualitative cell division takes place among the early oogonial and oocytal cells. His cytological accounts of the ovarian cells do not include the phenomenon of the polar body formation. Nevertheless, the most interesting feature of the article is the discovery of the symbiotic organisms in the egg as well as in the epithelial cells.

As the foregoing brief survey of literature indicates, two phases only of the development have been confirmed. The rest of the accounts still remain to be confirmed or rejected, while the origin and subsequent history of the pseudovitellus and the Pseudonavicellae demand a new and careful investigation. Again the development of several organs (respiratory, circulatory, sensory, and secretory) remains entirely undescribed.

The purpose, then, of this work is to contribute as much as possible toward the embryology of certain scale insects, with, however, especial reference to the history of the pseudovitellus, the germ cells, germ layers, alimentary canal, and nervous system.

Before going further, I take this opportunity to acknowledge my indebtedness to Professor Haseman, of the University of Missouri, with whom the work was carried on. My hearty thanks are due to Mr. Hollinger, who not only helped me in the collection and identification of the material, but also gave valuable information, and, above all, daily encouragement; and to Mr. Severance, of the library, through whose effort many valuable journals in the library of Congress and of other institutions were made available to me. Last, but not least, obligation is due to Professor Woodworth, of the University of California, with whom the study of the cottony cushion scale was originally begun.

Material and methods

Three species of Coccidae belonging to three different genera were chosen for the present investigation. These are: the mealy bug, Pseudococcus mcdanieli Hollinger (ms.); Hunter's Lecaniodiaspis, Lecaniodiaspis pruinosa (Hunter); the cottony cushion scale, Icerya purchasi Mask.

The material of the mealy bug was obtained from its most favorite host plant, the ragweed (Ambrosia trifida Linn.), on two trips in the latter part of September and another in early October, while that of Lecaniodiaspis was collected from time to time on two elm trees on the campus of the University of Missouri, during the season of 1917 to 1918. The cottony cushion scale was collected from Acacia and Pitosporum found in the vicinity of San Francisco Bay, California, during the seasons of January, 1915, to May, 1917.

Of the three species each had its own advantages. The eggs of the mealy bug were very easily fixed, sectioned, and stained, but they were so small that it was difficult to dissect away the chorions. The eggs of both the cottony cushion scale and Lecaniodiaspis are large, and the chorion can be removed easily. The eggs of the Lecaniodiaspis, however, stain with considerable difficulty. In fact, the egg of the cottony cushion scale was the most favorable material, having none of the disadvantages above mentioned. Yet it must be said here that it was with the study of Lecaniodiaspis and to a considerable extent with that of the Pseudococcus that the writer was able to see the true significance of several organs and inclusions.

The experimental method of determining the age of the embryo was tried to a considerable extent with the cottony cushion scale. For this purpose, about thirty adult females with the egg sacs were collected, together with the infested twigs. After removing the egg sac with a sharpened bamboo stick, the females were placed in small paper boxes. Every five or ten minutes the specimens were observed, and if an egg was seen protruding from the vaginal orifice, it was transferred into a numbered gelatin capsule. In the capsule, the egg was able to develop even to the

time of hatching. Thus the experiment went along with promise of success until several of the eggs supposed to be of the same age were fixed and mounted in toto. The examination of these prepared specimens, however, showed that no two of them were in the same stage of development. One of them contained an embryo nearly ready to hatch, another had an embryo with its appendages well recognizable, while the remainder were mostly in much earlier stages.

Sectioned material of several adult females of both the mealy bug and cottony cushion scale brought to light the fact that the eggs of these two species of coccids undergo a partial development in the uterus of the female. The early deposition of the egg was usually noticed in specimens in which the growth of the ovarian eggs was in rapid progress. The eggs were not deposited until the completion of the blastoderm. The eggs of the Lecaniodiaspis, on the contrary, were deposited at the first cleavage stage, and should serve as the most desirable material for this purpose. Unfortunately, I have failed to work with this species during the past year.

Thus the determination of the age of the eggs by the experimental method alone is not reliable. Therefore the relative ages of the embryos in this study were mostly determined by the number of cells, the position of the polar granules, the length of the embryo and of the appendages, and other morphological features.

Most of the material for embryological study was obtained from egg-sacs of the fully matured female scales in the following manner: Several females with their egg-sacs were collected at various times of the day, and the egg-sacs were separated from the body with a sharpened bamboo stick or needle. In some cases the eggs were lightly shaken out of the egg-sac into a watch-glass containing the fixing fluid, but in many cases the entire egg-sacs were dropped directly into the watch-glass containing the fixing fluid, and the cottony substance was removed afterward with a pair of sharpened bamboo sticks. The use of sharpened bamboo sticks proved to be advantageous, for they can be made of any desired sharpness and they are not acted upon by such corrosive mixtures as Gilson's.

One of the fixing reagents most extensively used was Carnoy's aceto-alcohol-chloroform mixture, prepared by mixing thoroughly equal parts of absolute alcohol, glacial acetic, and chloroform saturated with corrosive sublimate. Eggs fixed in this mixture for from one to two hours lost their red pigment and became transparent. They were then washed in 30 per cent alcohol for two hours, and passed through 50 per cent to 70 per cent alcohol with an intermission of one hour. They were then either left in 70 per cent alcohol until needed or dehydrated by passing them up through 90 per cent, 100 per cent alcohol to xylol and imbedded in 52° to 58° paraffin.

Sections were cut from 5μ to 7μ in thickness and stained mostly with iron alum haematoxylin followed by eosin, orange G, acid fuchsin, or a mixture of these. The triple stain, saffranin-gentian-violet, and orange G, was also frequently used with very good results.

For whole mounts, the eggs were passed from 70 per cent to 30 per cent alcohol, in which the chorion was dissected away under a binocular microscope. Embryos thus freed of their chorions were stained with a diluted solution of gentian violet for from one to six hours and then decolorized with 70 per cent alcohol. Delafield's haematoxylin, borax carmine, and alum cochineal have also been used with fairly good results. The most beautiful specimens, however, were those that were treated with gentian violet.

For the study of the history of the germ cells not only the genital organs of the embryo, but also those of several stages of larvae, pupae, and adult scales were necessary. Ovaries were mostly dissected out and fixed in either Fleming's or Zenker's solution. In many cases, however, whole larvae, pupae, and adult were put directly into Gilson's or Carnoy's aceto-alcohol-chloroform solution, sectioned and stained in the same manner as in the case of embryos.

Perenyi's solution was also tried, but all except the aceto-alcohol-chloroform mixtures were useless unless heated to 70°C., because the eggs as well as larvae are covered with a waxy substance which prevents penetration of fluids. When heated, these

fixing fluids as well as ordinary water will kill and fix the specimen, but in such a case the finer details of nuclear structure are often destroyed or distorted.

2. THE ORIGIN AND DIFFERENTIATION OF THE OVARIAN ELEMENTS

The germ cells in the ovaries of the larvae at the time of hatching are similar in size and appearance (fig. 1). By mitosis, these germ cells multiply during the first, second, and third larval stages and form a mass of so-called oogonia (fig. 6.) At an early period in the fourth larval stage, however, all oogonia cease to multiply. Consequently, all appear alike on account of their being in the so-called resting stage. This condition is soon followed by a peculiar phenomenon. A few oogonia situated along the periphery of the ovary suddenly undergo another, the last, oogonial division and begin to grow, not only in size, but also in nuclear complexity. The number of these oocytes of the first order forming a group varies with the species. In Pseudococcus there are four oocytes in a group, but in Icerya the number is five. At first the nuclei of the oocytes in each group appear exactly the same, all being in the so-called synizesis stage (fig. 4). From the contracted nuclear contents fine thread-like chromosomes emerge (fig. 6). At first these chromosome threads are distinctly doubled, but later appear as single.

Meantime a sort of protoplasmic substance begins to be secreted around each of the oocytes except the one situated toward the proximal end. As their later history shows, these secretory oocytes nourish the single oocytes located below or toward the distal end; and thus become the so-called nurse cells. The cytoplasm areas of the fast-growing nurse cells soon come into contact with one another. Being colloidal in nature, the nutritive substances secreted by the nurse cells elongate in the direction of the least resistance, which in this case is toward the egg nucleus, for the expanding force of the nurse cells is much greater than that of a single egg cell situated near the distal end. Consequently, the nutritive substance, which is elaborated by the nurse cells, now literally pours over the egg, causing a rapid increase in its size (fig. 11).

Epithelial cells which surround the nurse chamber above never multiply, but those around the egg multiply rapidly and help to accommodate the protoplasmic substance which pours from the nurse chamber above (fig. 15). Soon a constriction becomes evident at the junction of the two chambers (fig. 16), due partly to the ingrowth of the epithelial cells at the base of the nurse chambers and partly to the rapid expansion of the egg and nurse cells. The epithelial cells surrounding the egg chamber are cubical or elongate ovoid in shape and actively divide, while those around the constriction are smaller and spindle shaped. No mitosis was observed among the latter.

As the constriction progresses, the space through which the two chambers communicate becomes smaller. Consequently, the protoplasmic substance, which has been flowing homogeneously toward the egg nucleus or the germinal vesicle, now flows out of this small passage in minute streamlets as molasses does when poured through a funnel into a flask. The nuclei of the nurse cells still increase in size, and change from spherical to egg-shape, the narrow end being directed toward the egg chamber. The chromatin threads become broken into numerous chromatin bodies of different sizes. The nucleus of the egg proper moves toward the center of the egg chamber, but the chromatin threads are still in the paired condition as last described. Their affinity for iron-alum haematoxylin is changed. They now stain so faintly with this dye as to seem almost achromatic in nature. From this time on, the germinal vesicle begins to migrate from the center toward the periphery of the egg. During this migration the chromosomes lose their paired appearance and form several small, spherical bodies which become scattered along the nuclear membrane (fig. 17). There is, however, no indication of their being passed out through the nuclear membrane into the surrounding protoplasm, as several investigators of other insects have stated.

The place where the germinal vesicle reaches the peripheral layer is on the ventral surface, midway between the equator and the posterior pole of the egg. As soon as the clear transparent nucleus reaches the periphery, the surrounding protoplasmic

substance becomes compact, thereby causing an indentation of the surface.

In the next stage, the nuclear membrane of the germinal vesicle disappears and the vesicle itself loses its clear appearance. This change is due to the appearance of the spindle fibers about the chromosomes (fig. 26). The oocyte is now in the metaphase of the maturation division. Following this stage the chromosomes divide into two groups and move to the opposite poles. Soon one of the daughter cells gradually protrudes from the egg proper (fig. 31). In this manner the first polar body is formed. The process of the second polar body formation has not been studied, but that the egg undergoes the second maturation division may be established by the presence of three polar bodies. Meanwhile the epithelial cells not only cease to multiply, but they also become reduced to a membranous structure. The nurse cells also cease to grow. Their size becomes very much reduced. The contents of the nurse chamber is withdrawn into the egg and its epithelial layer shrinks to a small mass. This, together with the remains of the epithelial cells of the egg, closes over the opening left by the egg entering the oviduct.

The earliest egg found in the oviduct (or the uterus, as it is often called) shows a large nucleus at the center of the egg. Since this large nucleus divides, it must be the first cleavage nucleus. It follows, therefore, that the union of the male and the female pronuclei must have occurred during the passage of the egg pronucleus to the center of the egg after the formation of the last polar body.

Thus my observations on the three species of coccids are in accord with those of Leuckart ('53) and Emeis ('16). It may also be added that the ovary of Icerya purchasi is the most favorable material for the study of this problem, since the cells are large and numerous.

The origin of the three ovarian elements in aphids has been differently described by different writers. Lubbock claims that the egg and nurse cells are modified epithelial follicular cells of the end chamber. Recent observations of Tannreuther ('07) are to the same effect, for he declares that the egg cells do not arise

from the inner mass in common with the nurse cells or ovarian glands, but grow out of the follicular epithelial cells at the base of the end chamber.

Balbiani ('82) states that the germ rudiments of parthenogenetic aphids undergo a process of budding previous to their differentiation into the nurse cells and oocytes.

Mecznikow ('66) derived the end chamber from a mass of cells, "Die am untersten Pole des endfaches liegenden Zell sich bedeutend vergrössert, wobei sie in ein, aus dem Endfachepithel entstandes Follikel eingeschlossen wird und hier ihre weitere Entwicklung vollzieht."

Stevens ('05) insists that the contents of the end chamber are of two kinds, possibly corresponding to the summer and winter eggs, and that those situated on the lower portion of the end chamber degenerate in the ovaries which produce the agamic eggs.

In insects (other than Hemiptera) the results of several investigators differ with the species with which they have worked. The prevailing idea, up to 1905, was that the three ovarian elements, nurse cells, epithelial cells, and oocytes, are all derived from the germ cells. Paulcke ('00) who studied the development of the honey-bee, however, discovered for the first time that the nurse cells and oocytes are originally the same, but later become differentiated by a certain irregular cell division. Similar discoveries of the existence of a quantitative difference between the nurse cells and oocytes have since been reported in several Coleoptera. In Dytiscus, for example, Gunthert ('10) found that the chromatin eliminated from the nucleus passes, in each successive mitosis, into the pole of a single daughter cell, and that the cell having this extra chromatin substance becomes the oocyte and those lacking this the nurse cells. Somewhat similar observations were made by Giardia ('01) and Debaisieux ('09) in Dytiscus, and Govert ('13) in Carabus, Cicindela and Trichiosoma.

The oogonial origin of the oocytes, nurse cells and follicular epithelial cells was clearly established in Polistes and Platyphylax by Marshall ('07); in Podura by de Winter ('13), and in Leptinotarsa by Hegner ('14). In these cases, the nurse cells

and follicular epithelial cells are regarded as abortive cells. No differential mitosis was observed.

Hegner ('12) states that the germ cells in Miaster give rise to nothing but the true oocytes, and that the nurse cells and epithelial cells are both derived from somatic cells. He deduces this from the fact that in Miaster americana altogether sixty-four oogonial cells are formed by six successive divisions of a single primordial germ cell and that the number of the young larvae produced is also about sixty-four.

The foregoing survey of the more important literature pertaining to this subject, brief as it is, indicates that, even among the same order of insects, there is no definite law governing the differentiation of the three ovarian elements. The accounts of the lineage of the ovarian elements in Leptinotarsa (Wieman, '14; Hegner, '12), and Hydrophilus (Korschelt, '89) are good examples. However, it should be mentioned that in all insects the oocytes and also the nurse cells, when present, are all derived from primordial germ cells. As yet no case has been found in which the primordial germ cells of the insects entirely degenerate and the secondary or functional germ cells are formed de novo at a much later period of development.

3. THE EGG

The eggs of all species of coccids studied, at the stage last mentioned, consist of the following substances:

1. Chorion—the outermost covering or membrane.
2. Protoplasm—the ground substance.
3. Corticular layer—a thick protoplasmic layer next to the chorion.
4. Fat globules—oily droplets suspended in the protoplasmic network.
5. Yolk granules—protoplasmic suspension.
6. Pigment oil-fluid filling interspace between fat globules.
7. Germinal vesicle with its nuclear membrane.
8. Yolk membrane—membrane next to, and, in fact, almost apposed to the chorion.

The chorion is a very thin membranous structure which encloses the substances above mentioned. It is formed shortly before the passage of the egg from the egg chamber into the uterus, and is secreted by the follicular epithelial cells.

The protoplasm or cytoplasm fills, so to speak, most of the space between the other inclusions of the egg, with the exception of the space occupied by the nucleus or the germinal vesicle. As already stated, this ground substance of the egg is elaborated by the nurse cells and is literally poured on the egg. At first the protoplasm is a homogeneous mass uniformly surrounding the central clear region, the nucleus. Later, however, it becomes mesh-like, owing perhaps to the more rapid expansion of the egg than the flow of the nutritive or protoplasmic substance from above, to the intrusion and consequent suspension of other substances, and also to the physiological change due to the metabolic activity of the germinal vesicle.

What seems to me a sort of yolk substance is found in the egg of the mealy bug of the giant ragweed. This substance may be spherical, but it is more often irregular in shape. It first appears at about the time when the germinal vesicle reaches the periphery. The exact origin of this substance remains to be studied further. The fact that similar granules are abundant in the body cavity surrounding the ovarioles, and also in spaces between the chorion and the epithelial cells, strongly suggests that these particles may actually migrate from the body of the mother through the epithelial layer into the egg. The presence of similar substances in the body cavity of the mother is another evidence in favor of the view just stated. Several investigators of other insect eggs state that they have observed the migration of chromatin matter from the germinal vesicle of the egg. No indication of such migration was observed in the case of the scale insects studied.

In the ovarian eggs of these species of coccids, the nucleus or the germinal vesicle was always found.

The pigment-oil or coloring matter appears in the mature, but not in the ovarian egg. I have had occasion to observe this fluid-like matter flowing into the egg at the posterior or pointed

end. This substance was found literally filling the oviducts of the adult during her egg-laying period.

The presence of the yolk membrane cannot better be illustrated than by figure 28. On account of the migration of the symbiotic organisms after the formation of the yolk membrane, the latter is pushed in and remains separated from the chorionic membrane (which is the last to envelop the egg).

A fully matured egg with all its components is elongate oval. The pointed end corresponds to the cephalic and the blunt end to the caudal end of the insect. The ventral surface near the pointed end is slightly indented. Thus not only the anteroposteriority, but also the dorsoventrality are marked in the eggs of the coccids, but not so clearly as in the eggs of the Orthoptera and Coleoptera reported by Heymons ('89), Wheeler ('93), and others.

Besides such a difference in shape, the anteroposteriority is well marked by the presence of a dark-staining substance, the position of which varies with the species. In cottony cushion scale, it is, at first, visible near the posterior pole, but later becomes pushed gradually toward the anterior pole by the invaginating germ-band; while in the case of Pseudococcus and Lecanodiaspis, it is found always near the anterior end of the egg. The presence of these polar granules or symbiotic organisms is a great service in the determination of the position of the sectioned material. Later on, I shall treat of the history and significance of this substance under a separate heading.

No micropyle was found.

The longest and shortest diameters of the eggs of the three species of coccids are respectively as follows:

NAME OF SPECIES	LONG DIAMETER	SHORT DIAMETER
	mm.	*mm.*
Icerya purchasi	8.5–9.0	4.5–5.0
Pseudococcus macdanieli	4.0	2.5
Lecaniodiaspis pruinosa	6.0–4.0	2.5

4. CLEAVAGE

The type of cleavage in the coccids studied is the pure superficial type which is so common among the Arthropoda. The first cleavage spindle lies at right angles to the shorter axis of the egg, so that one of the two daughter cells arising from the first division wanders toward the posterior pole while the other cell remains near the position formerly occupied by the mother nucleus (fig. 43). This behavior of the first two cleavage cells in coccids is exactly like that of the termite studied by Knower ('00). At first, all the cleaving cells were in the same mitotic state, but gradually some lag behind others in division so that in a later stage of cleavage, e.g., at the thirty-two cell stage, more than one cleavage figure is noticeable among them (fig. 78). Up to about the eight-cell stage in the eggs of Lecaniodiaspis, and to a still later stage in Pseudococcus and Icerya, these cleavage cells are all at some distance from the cortical layers. Although in each cell the nuclear membrane is distinct, the cytoplasm presents numerous pseudopodial processes which connect with those of neighboring cells. On account of their somewhat isolated appearance, they are usually known as protoplasmic islands. In eggs containing a large amount of yolk, as, for example, those of Chrysomelid beetles studied by Hegner ('14), the winter eggs of plant-lice investigated by Tannreuther ('07) and Webster and Phillips ('12), these protoplasmic islands literally cut up the yolk into blocks. I have noticed this block-like appearance of the egg contents in the living eggs of Lecaniodiaspis, but upon sectioning them, I become convinced they were not comparable to the yolk-blocks found, for example, in the ova of aphids, because the eggs of Lecaniodiaspis contain no yolk granules. The eggs of the mealy bug contain a darkly staining substance resembling the yolk of the ova of aphids, but they are never cut up into blocks by the cleaving cells (fig. 42).

Weismann ('82) stated that in Rhodites and Biorhiza aptera (cynipids), the first two cleavage nuclei move apart in the direction of the longitudinal axis of the egg. One of them, upon reaching the posterior pole of the egg, remain inactive and probably degenerates, while the other, upon arriving at the anterior pole,

produces, by rapid multiplication, all of the embryonic cells. As stated above, the first cleavage products of our scale insects do not behave in this way, but both cleavage cells continue multiplying, and some of their products later form the blastoderm, while the others remain in the interior of the egg and constitute the so-called yolk-cells. In this respect, the development of the eggs of coccids resembles that of the silkworm and of Neophylex and Gryllotalpa, studied, respectively, by Toyama ('02), Patten ('84), and Korotneff ('84). Silvestri ('11) recently discovered that in a parasitic Hymenopteran, Copidosoma, one of the two nucleoli escapes from the nucleus at the end of the growth period of the oocyte. Later, this escaped nucleolus passes into one of the two cleavage cells. During a series of cleavage processes, only one cell remains in possession of this nucleolar substance and becomes the germ cell. In another parasitic Hymenopteran the escaped nucleolar bodies become localized at the posterior end of the egg until one of the first cleavage cells reaches out and takes them up into its protoplasm. In both cases the cell which becomes possessed of this escaped nucleolar substance, differentiates into the germ cells.

In the scale insects I have studied no escape of the nucleolar substance into the egg was observed and the germ cells do not appear during the cleavage period.

All cleavage cells divide mitotically. No case of amitotis, as described for Blatta by Wheeler ('93), has been observed. The fact that very many cells are in the process of division during the early stages indicates the rapidity with which cells divide. Nelson ('15) states that no case of a single spireme stage was found in the cleavage cells of the honey-bee. On this point my specimens agree strictly with his observation. An abundance of spireme figures are, however, found among the blastoderm cells. As the number of cleavage cells increase, they migrate, one by one, toward the periphery and become imbedded in a thick cortical layer of the protoplasm. In figure 44 the condition of a loose blastoderm is shown. Although the cells are arranged in a peripheral layer, they are very far apart from one another. The spaces between these blastoderm cells are gradually filled by the

division of the blastoderm cells as well as by a further migration of cleavage cells from within.

The point at which cleavage nuclei, or cells as they are often called, reach the surface of the egg varies in different groups of insects. In Muscidae Graber ('79) found the first arrival of cells at the posterior end of the egg, while in Pieris Bobretzky ('78) observed the appearance of the first blastoderm at the anterior end. Wheeler ('93) described the first blastoderm cells on the ventral side, while Heider ('88) stated that the blastoderm in Hydrophilus was first formed around the middle of the egg as a transverse girdle, somewhat nearer the posterior pole and that the development occurred last at the poles. Again, according to Nelson, the cleavage cell first reaches the cortical layer on the ventral side near the cephalic pole in the egg of the honeybee. In the winter egg of the aphid, Melanoxanthus (Pterocomma salices), according to Tannreuther ('07), all of the blastodermic cells spread uniformly over the entire surface except at the posterior pole of the egg. Therefore, I agree with Nelson ('15) that the point at which the first cleavage cells reach the surface has little significance so far as the formation of the blastoderm is concerned.

The condition of the egg at the time the cleavage process has ceased among the cells within the egg and the blastoderm formation is completed, is shown in figure 80. At the poles and sides the blastoderm is similar in appearance. A short distance within the blastoderm is another loose layer of cells. This is irregular in shape, and the nuclei are much clearer and coarser than those of the blastodermic cells. These are the so-called yolk cells of Will ('84), and are no other than the cleavage cells that failed to migrate to help form the blastoderm. At about the time invagination occurs at the posterior end of the egg, these cells move toward the periphery and become closely apposed to the blastoderm cells.

5. ESTABLISHMENT OF THE EXTERNAL FORM OF THE EMBRYO

The development of the embryo was traced up to the completion of the blastoderm as shown in figure 80. I first describe the development of the embryo as seen mostly from surface views.

The first change externally visible after the completion of the blastoderm is a depression or an invagination near the posterior end of the egg. It is, at first, very shallow, but gradually deepens forming a V- or U-shaped structure (fig. 45). This condition is much more pronounced in the case of the cottony cushion scale than in the other two species studied. The portion of the blastodermic layer constituting the bottom of the blastopore and its near-by area increases greatly in thickness, while toward the anterior pole and in the area surrounding the blastopore it becomes thin. In the cottony cushion scale, the colony of parasitic organisms, originally found at the posterior pole of the egg, is later pushed, so to speak, toward the anterior pole by the elongation of the invaginating germ band.[2] During the same period of development, the mass of the parasitic organisms in the eggs of Pseudococcus and of Lecaniodiaspis has also migrated a short distance from its point of entrance, the anterior pole, towards the posterior pole. A side view of a similar embryo (fig. 52) shows that the invagination occurs, not exactly at the posterior pole, but a short distance lateral to it. The same figure also brings out the fact that the layer forming this depression and also the ventral germ band is essentially a continuation of the outer blastoderm, a portion of which forms the serosa. Only the lower, or the ventral portion of these, later becomes the embryo, while the upper or the dorsal wall transforms into the amnion. A later condition of the egg is represented in figures 59 and 62. The invaginating germ band is now about two-thirds as long as the egg. In the side view of a slightly older embryo the germ band appears as though it con-

[2] In this paper the term germ band is used to designate the ventral wall of the invaginated embryonal rudiment in its early stage. The term ventral plate denotes the same structure after the amnionic layer becomes clearly distinguishable as the dorsal wall of the invagination.

sists of two bands separated by the median groove. In reality, however, the furrow represents the amniotic cavity, the blastopore, within the invaginating germ band. The colony of parasitic organisms was not, at first, in contact with the germ band, but it now appears as though it were in the same relative position as in the case of the cottony cushion scale.

As the invagination proceeds further, the caudal portion of the invaginating germ band curls up ventrally, assuming the shape of the letter 'S.' Each turn of the invagination represents a particular region or division of the body of the embryo as should have been brought out earlier. The germ band at the stage last described consists of four main divisions, representing the cephalic, the oral, the thoracic, and the abdominal regions. (figs. 46, 50, and 64). These regions are shown in figure 6, separated by three dark areas. The two remaining regions cannot be shown very well in the picture, but their presence is represented. Each dark area indicates the junction of two adjacent regions.

It should also be mentioned that the preoral and abdominal regions are much less extensive in length as compared with the oral or thoracic region. The colony of parasitic organisms, which had been located near the growing tip of the germ band of the cottony cushion scale, now becomes fixed, so to speak, at the region of the second and third abdominal segments and does not accompany the growth of the abdominal region beyond these segments. The condition described for the cottony cushion scale is accomplished in an altogether different manner in the case of the mealy bug and Lecaniodiaspis. Instead of pushing the colony of parasites forward, the germ bands of these two species sends out a few germ cells at first, then follows a mesodermal extension to the colony, which establishes a relation with the latter, just as in the case of Icerya. During this stage a pair of somewhat curved, dark elevations appear, one on each side of the germ band near the blastopore. These, as will be seen later, are the rudiments of the brain. In the next stage (fig. 66), the first rudiments of appendages, the second maxillae, become apparent. The abdominal region is very much elongated beyond the colony of parasitic organisms. The division of

the body into four regions becomes plainly marked. A lateral view of a somewhat older embryo is shown in figure 61. Here the abdominal region is so much elongated as to be actually folded over beyond the thoracic part, and reaching almost to the base of the second maxillae. The brain becomes very conspicuous, owing to its enormous dorsal growth. Below the rudiment of the brain another pair of elevations appears (fig. 65). This is the rudiment of the first maxillae. Segmentation is clearly visible throughout the oral, cephalic, thoracic, and the abdominal regions. In a still older embryo (fig. 67) three pairs of appendages become visible. These are the rudiments of the thoracic legs. Thus the appendages of scale insects, like those of most other insects, develop first from the maxillae backward to the last thoracic limb and finally to the antennae and mandibles, the labrum being the last to appear. In other words, the rudiments of the second maxillae are the first to appear. The first maxillae and the thoracic legs make an almost simultaneous appearance, followed by the antennae, mandibles, and the labrum. In this respect, the scale insects differ from the aphids, in which, according to Mecznikow and Witlaczil, the antennal appendages are the first to appear. The order of the appearance of the appendages in Pseudococcus and other scale insects differs also from that observed in the Orthoptera in which, as described by Wheeler ('89), Riley ('98), and others, the antennae are the first to appear, followed by oral appendages, the thoracic limbs being the last to be formed. My observations also differ from those made by Brandt on Libellulids, when the rudiment of the thoracic limbs appears first, then those of the maxillae; the antennae being the last to develop. In Coleoptera, i.e., Hydrophilus, Melolontha, etc., according to Heymons and others, the antennal rudiment seems to appear first, while the mandibular and maxillary rudiments and those of the thoracic legs make a simultaneous appearance.

In Mantis (Hagen, '17) the antennal rudiments appear first, followed by those of the thoracic appendages; then appear, practically simultaneously, the rudiments of the maxillae and labrum, the last being distinctly an unpaired organ.

The further growth of the appendages is shown in figure 47, which represents the surface view of a somewhat older embryo. Mandibles, maxillae, and three pairs of thoracic legs are now tubular instead of conical elevations. The second maxillae have migrated somewhat dorsally and therefore are no longer in line with the rest of the appendages, as was also noticed by Mecznikow ('66) for Lecanium. The three pairs of thoracic legs appear somewhat constricted at the middle, indicating that they are, in a sense, two segments.

The first rudiment of the mouth is found in figure 49. This is essentially a circular depression of the ventral plate in the medial line between the rudiments of the brain elevations and is cephalad of the oral or antennal appendages.

Following this stage, the brain undergoes a conspicuous development. Heretofore the rudiments of the brain were only represented by two crescentic elevations, one on either side of the median line. It now becomes a large conical structure, due to its dorsal turning and rapid neurogenesis. It, however, lies entirely within the serosa sac and not beyond the original 'blastopore' of Will, as he maintained was the case with the development of the pseudovum of aphids. The brain region is also surrounded, at least ventrally, by the amniotic layer. That the rudiments of the antennae arise, not from the posterior end of the brain, but from below it, is also clearly demonstrable here. The segmentation of the abdominal region into its future body segments is also clearly shown.

In the next older embryo (fig. 95) several notable changes are evident. The first maxillary rudiments have migrated still further dorsally, leaving their alignment with the rest of the appendages. The three pairs of thoracic limbs now present a slight constriction approximately at the middle of the appendages. The segmented abdominal region is still folded over the thoracic as well as the oral regions.

Following this stage, the embryo gradually shortens until it begins to rotate around the transverse axis of the egg. As a result of this rotation, the poles of the egg coincide with those of the embryo (figs. 53, 72). Heretofore, the cephalic or anterior

end of the embryo has been situated at the posterior pole of the egg because of its being the part first formed from the invaginated portion of the germ band which occurred at or near the posterior pole of the egg, but now it has accomplished its exchange of poles by the process of revolution just mentioned.

A side view of an embryo after its revolution has been completed is shown in figure 53. The thoracic limbs are now distinctly four jointed, showing the coxa, trochanter, femur, and tibia. The segments of the limbs are almost equal in length, but differ in shape, the second being the largest and the last the most slender. The antennae also have four segments. The most conspicuous feature at this stage is the appearance of the deep invagination of the proctodeal opening on the dorsally curled end of the abdominal segment. The proctodaeum invaginates on the dorsal side of the ninth abdominal segment as will become clear when its formation is studied in sections. Another interesting feature of this stage is the sudden appearance of a mass of cells on the dorsal side of the brain segment. In reality, however, this mass of cells is formed during the revolution of the embryo by the contraction of serosal and amnionic cells. Another prominent change noticeable at this stage is that which occurs in the formation of the mouth parts.

The mandibles and the first maxillae in the stage last described have migrated laterally and are no more in line with the second maxillae. A large invagination between the second maxillae and the first thoracic legs appears during the revolution of the embryo around the shorter axis of the egg, and there arises, from the pointed distal end of each of the mandibles and the first maxillae, a slender chitinous bristle-like structure, which is from the beginning non-cellular. The wall of the invagination, on the contrary, is cellular at first, but later becomes chitinous. The cavity thus formed and lined with a chitinous layer, it must be mentioned, serves to accommodate the bristles when they are not in use, as when the larvae changes its feeding place.

The upper lip, with a well-defined suture, does not elongate or grow very much in size, but a layer of chitinous substance is

secreted all over the surface forming a semiconical structure above the base of the mouth parts. The second maxillae, which also remain relatively small, are also invested with the chitin, and finally form an inverted crescentic underlip. The suture formed along the midventral line by the fusion of the second maxillae remains open externally and also opens internally into the ventral cavity. It is through this external opening that the bristle-like piercing organs pass out at the time of feeding.

The formation of the mouth parts of the coccids is essentially the same as that given for Corixa by Mecznikow ('66), who says:

> Die Mandibeln und die ersten Maxillen liegen jederseits dicht beisammen, die Form kleiner Zapfen zeigend. Die zweiten Maxillen verwachsen mit ihren unteren Rändern, wodurch eine mächtige Unterlippe zu Stande kommt. Die oberen unverwachsenen Ränder der beiden Maxillen bleiben und repräsentiren die bekannte Rinne der Unterlippe.

The same investigator, however, gives a different account of the formation of the mouth parts in aphids in the following words:

> Sie (the beak) werden von besondern Körper secernirt, welche jederseits neben den Mandibeln und Maxillen im Laufe der dritten Entwicklungsperiode entstehen. Bei ihrem Wachsthum nehmen diese Körper bald eine retortenförmige Gestalt an, wie es auf der Fig. 53 abgebildet ist; es schnürt sich dann von ihnen eine dünne peripherische Schicht ab, welche das Licht stärker bricht und, sich verlängerned, einem schmalen Faden den Ursprung giebt. Es entstehen somit jederseits zwei solche Fäden, welche nunmehr in die fraglichen Stillette übergehen und dabei die Fähigkeit erhalten, nach aussen in die Rüsselscheide ausgestülpt werden zu können. Die Retortenform der beschriebenen, aus einer Menge kleiner gleichgestalteten Zellen bestehenden Körper verursacht es, dass im ruhigen Zustande das fadenförmige Stillette spiralig aufgerollt liegt.
>
> Bei weiteren Wachsthum der Mündanhänge nehmen diese an Länge zu, wobei man an der ersten Maxille die erste Bildung eines Tasters wahrnimmt. . . .

Mecznikow must have had the idea that the mouth parts of the coccids are formed in the same manner as in the case of the aphids, for he states at length the difference between the formation of the mouth parts in the Heteroptera and Homoptera. My

observation on several coccids shows, it should be repeated, that the mouth parts of these coccids (Homoptera), are formed essentially in the same manner as in Corixa, a Heteropteran.

The side view of the last embryonic stage is shown in figure 56. In each of the thoracic limbs, the first joint or coxa becomes conical. Contraction has also considerably reduced the length of the trochanter. The embryo is now shortened to the length of the egg-shell and is much flattened. The alimentary canal is already completed, and the ventral nerve cord is contracted a great deal. A great change has also occurred in the oral appendages. The tips of the mandibles and first maxillae become elongated, extending beyond the rest of the oral appendages to form slender lancelet-like processes, while the rest of the oral appendages unite and form a sort of case at the base of the piercing processes. A careful examination of a mounted specimen of this or a slightly older embryo shows that the antennae are covered with a long slender hair-like process at each joint. There are also tail filaments, three on either side of the posterior extremity of the tenth abdominal segment. The embryo is now ready to hatch.

In the case of the cottony cushion scale, I have observed the process of hatching. The first indication of this process is the appearance of a transverse slit, usually around the mesothorax, often, however, on the metathorax. Through the rent thus formed, the antennae, which have been folded against the ventral surface of the body, gradually stretch out. By the force thus exerted by the antennae the cephalic portion of the insect becomes first lifted and then carried forward from the egg shell by the stretching antennae, meanwhile the embryo juggles its abdominal portion by exertion on the part of the long caudal filaments. Then the femur of the first thoracic leg and a part of the first tibia appear. With the next exertion the most of the body and legs become free from the egg shell. The rest of the process is the kicking off, so to speak, of the shell from the filaments by means of legs, and the hatching is completed.

The embryo, eight hours after hatching, is represented by figures 57 and 75, of Pseudococcus and Icerya, respectively.

6. THE FORMATION OF THE GERM LAYERS AND THE EMBRYONIC ENVELOPES

At the time the invagination occurs at the posterior end, the blastoderm is one cell thick, except at the posterior end where cell proliferation is evident. The yolk cells (cleavage cells that failed to take part in the formation of the blastoderm) are here and there closely attached to the blastoderm. Now and throughout the embryonic period no evidence of cell migration from the embryo proper into the yolk is noticed.

The first change occurring after the completion of the blastoderm is its thinning out in the immediate neighborhood of the posterior pole and also toward the anterior end of the egg, and the simultaneous thickening around the rim of the thinned area at the posterior end of the egg. The thickening near the posterior end is due to the proliferation of cells, as shown in figure 81. The proliferating cells at first are all similar, as in figure 83. The larger and clearer cells found in chains, from the region of the invagination toward the opposite end of the egg, are the germ cells, the history of which will be considered separately. At this stage, then, there are only two kinds of cells within the blastoderm: the germ cells and the cells that form the invaginating germ band.

In figure 85, which represents a longitudinal section of a somewhat older embryo through the region of the blastopore, cells of another or third kind occur between the germ cells and the cells which form the wall of the blastopore. These are the so-called entodermal cells. The cells forming the wall of the invagination and situated next to the entodermal cell mass are cubical or almost columnar and are in direct continuity with the blastoderm layer beyond the invagination or the blastopore. These are the rudiments of the future ventral plate and amnion.

A longitudinal section through the blastopore of a similar embryo (fig. 86) shows another, a fourth sort of cells besides the three already mentioned. Although these latter cells may have been present in sections of the egg illustrated in the preceding figure, they have perhaps escaped detection on account of the syncytial nature of the cells at that period. It is at about the

stage of development here illustrated that the mesodermal cells become easily distinguishable from the other two cell layers, namely, the columnar ectodermal cells forming the floor of the invagination above and the layer of much smaller and feebly staining syncytial cells below. The mesodermal cells at this time can be distinguished from the ectodermal cells by their large size and coarsely granular nuclear contents. The staining reaction of the two is also different: the mesoderm stains heavily, the entoderm only very feebly.

Up to this stage, invagination, in the case of Pseudococcus, is indicated by a slight depression at a short distance ventrad of the posterior pole of the egg. As the invagination progresses further, the depression becomes deeper and its walls more closely compressed (fig. 85). Mention should be made of the fact that the depression in Icerya and perhaps in case of the other species is, at first, in the form of a cup with broadly rounded bottom. The entodermal cells are always found opposite the base of this cup-like depression. With the further growth of the embryo, the dorsal wall of the depression, now a compressed tube, becomes thinner and, at the time when the embryo reaches the anterior mass of the parasitic organisms, it becomes reduced to a layer one cell thick. The ectoderm cells also undergo a marked change. They are arranged in a layer beneath or ventrad to the amnion, and, at the same time, become much more elongated, with their nuclei arranged alternately, giving the appearance of two layers. The mesoderm, again, has spread out to its full length below the ectoderm, which is now the ventral plate. The cells of the ectoderm, mesoderm, and entoderm are still continuous at the extreme bottom of the invagination where the primitive condition still exists.

From this time on, the caudal or abdominal portion of the embryo elongates, extending over and above the ventral surface of the ventral plate. Consequently, any transverse section passing through the thoracic region also passes through an abdominal segment. Figure 103 shows such a section through the second maxillary segment and one of the abdominal segments. Here, in the ventral or thoracic section, the amnion appears as a

thin membrane composed of a few flattened cells. The mesoderm cells, which, on both sides, meet the amnion and ectoderm, form a layer one cell in thickness. Although the lateral extensions of the mesoderm have followed the evaginations of the appendages, the layer is still one cell in thickness. The dorsal or caudal section presents nothing new. It resembles a section through the thoracic region of a younger embryo. Both the amnion and the mesoderm consist of spherical cells, while the ectoderm cells are spindle-shaped. All three germ layers are continuous at the sides.

A longitudinal section of a somewhat younger embryo of Pseudococcus (fig. 99) and one of Lecaniodiaspis (fig. 89) show that the amnion actually terminates at both the caudal and cepahlic ends and that it incloses the parts of the embryo. The layer of mesoderm, which is much thicker at the caudal region, gradually thins out toward the oral region, the cephalic region being entirely devoid of it.

A transverse section of an older embryo (fig. 104) shows, among other things, a remarkable change in the mesoderm. On account of the neurogenic swellings, which occur on both sides of the median line, the mesodermal cells have been pushed aside, so to speak, from the area immediately dorsal to the neurogenic area and have been shifted to the sides where the evagination of the appendages has taken place.

A transverse section through the second maxillae of a much older embryo is shown in (fig. 107). The dorsal or caudal section shows, among other things, that the amnion is represented by a single layer of cells, and that the mesoderm has expanded, not only into the evagination of the appendages, but also in the dorsal extension of the ectoderm.

A comparison of figures 103 and 107 will show that the differentiation of germ layers takes place from in front backward, as in other insects, except the agamic aphids, in which, according to Will ('88), the cephalic portion develops earlier than the brain.

As the appendages further elongate, the mesoderm follows the ectodermal evagination, forming a double-layered structure.

Similarly, the mesoderm keeps pace with the dorsal extension of the ectoderm in the closure of the dorsal wall, as in the case of other insects.

As the brain gradually grows in size, the amnion, which is situated above, is pushed out until it comes into contact with the serosa, and a rupture occurs at the point of union. Through the rent thus formed, the embryo emerges from the amniotic cavity and begins to rotate around its transverse axis until the poles of the embryo coincide with those of the egg. During this rotation the serosa and the united portion of the amnion shortens. As a consequence, the cells that formed the serosa and amnion aggregate at the dorsal end of the brain as a dense mass of ellipsoidal cells—the dorsal organ. This dorsal organ lasts but a short time, for it soon comes into contact with the invaginating tip of the stomodaeum, where its elongate cells first become disarranged and finally disappear, probably being taken up by the cells of the stomodaeum.

The exact origin of the mesoderm cells is very difficult to determine in the case of Icerya and Pseudococcus, because of the crowded condition of the cells about the invagination pore. In figure 83 the germ cells and a few entoderm cells are seen, but no mesoderm cells. But in figure 86 there are four kinds of cells, namely, the germ cells, ectoderm, mesoderm, and entoderm cells. Therefore, mesoderm cells must have appeared during the period between these two stages. The way in which the mesoderm forms can, however, be well ascertained in the case of Lecaniodiaspis, where there are comparatively few cells in the early period of invagination. In figure 91 both the amniotic and the ectodermal cells, as well as the mesoderm cells, are all connected with the blastopore. This fact, I believe, indicates that not only the mesoderm, but also the entoderm cells are both developed simultaneously with the invagination at the same point, namely, at the tip of the blastopore of Will. In this regard, then, my observation differs from that of Witlaczil for aphids, where the mesoderm layers are supposed to arise from the ectoderm layer by delamination. Moreover, my interpretation of the mesoderm formation is in accord with that of Will

for aphids. With Will, I therefore conclude that, in these families of insects, namely, Aphididae and Coccidae, and perhaps others, there is a process of true gastrulation as in other higher animals, and that the invagination pore of our insects represents the blastopore of other animals, because it is here that the three germ layers arise.

Mention should also be made that, in the case of coccids studied, the mouth and the anus arise, respectively, at the anterior and posterior ends of the invagination. Accordingly, I agree with Will, Wheeler, Kowalevsky, and others that the blastopore of insects is homologous to that of other animals, e.g., of amphibia, and that it is much elongate, even to the extent of forming a loop within the egg.

As previously stated, the entodermal cells in Pseudococcus macdanieli become localized at the posterior end of the germ band only. My interpretation of this unique phenomenon is this: On account of scarcity of yolk, the entodermal cells remain in a more or less inactive state as compared with the rapidly proliferating mesoderm cells beneath. Consequently, the former become entirely cut off from the point of their origin, the blastoporic rim. If this interpretation be correct, it follows that the scale insects are much more specialized than the Orthoptera, in which, according to Wheeler ('93) and others, the entoderm is found at the two extreme ends of the germ band.

7. THE FORMATION OF THE NERVOUS SYSTEM

The nervous system of the coccids studied is formed exactly in the same manner as in the case of other insects, for example, in Blatta germanica, so well described by Wheeler ('93). No account, however, of neurogenesis in Hemoptera,—aphids, coccids, and others—has ever been presented, so some details of the history of the nervous system of the coccids may not be out of place.

Although the brain of the scale insects is but a continuation of the ventral cord, and the ventral cord, in its genesis, is but a part of the whole nervous system, the formation of these two parts will be considered separately, as has been customary among the embryologists.

A. The ventral nerve cord. The rudiments of the ventral nerve cord arise from the ectoderm layer of the ventral plate, the origin of which has already been described. At first the ectoderm cells forming the ventral wall of the amniotic cavity are all similar in appearance, size, staining reaction, etc. They are all cylindrical, almost spindle-like in shape (fig. 103).

Later, however, on each side of the long axis of the embryo, a few large spherical cells are formed within the ectoderm layer by mitotic division. Since, as will be shown later, all of the nerve cells arise from these large spherical cells, the name, neuroblasts, is applied to them. The remaining cells in the ventral ectoderm do not contribute to the nervous system, but go to form the body wall, and consequently they are called the dermatoblasts. In like manner, the term neurogenic area and dermal layer are, respectively, used to designate the portion of the area containing the neuroblasts and that without them.

The number of the neuroblasts increases by a further mitotic division of ectodermal cells. In figure 104 four such neuroblasts are shown, two on either side of the middle line, and in addition to these, a division figure of an ectoderm cell is given.

In figure 107, which represents a transverse section through the first thoracic appendages, the separation of the nerve rudiments from the dermal layer is pronounced. The ectoderm layer now becomes so thin that it is everywhere but one cell in thickness. On the other hand, the nerve rudiments are several cells thick. A layer of cells next to the dermal ectoderm in each of the body segments consists of six large cells. Each of these neuroblasts is usually seen in a different phase of mitosis. Dorsad to the neuroblasts, there are about five horizontal rows of smaller cells, which stain much darker than the surrounding neuroblasts, the dermatoblasts, or the mesoderm cells. Nevertheless, these darkly staining cells are the true daughter cells of the neuroblasts and are called ganglioblasts, for they form the ganglia. All ganglioblasts are of the same size and are in the resting stage. These facts indicate that they do not divide after they become once separated from the neuroblasts.

Comparisons and measurements of the neuroblasts in different stages of development show that they are true teloblastic cells. They do not diminish in size, but resume their original size after each of a series of cell divisions. Thus, in fact, there is no cell intermediate in size between the neuroblasts and the ganglioblasts. A comparison of figures 103 and 107 indicates that the development of the ventral nerve cord takes place from in front backward, as was the case in the development of the appendages already described.

The abdominal ganglia, the number of which corresponds with that of the segments, are noticeable in the embryo, a longitudinal section of which is shown in figure 118. The nerve cord in the figure is completely separated from the ectoderm of the ventral body wall in the cephalic as well as in the thoracic segments, but in a few of the last abdominal segments they are still connected. In fact, the separation of the ventral nerve cord, like all phases of differentiation, takes place from before backward. Each mass of ganglionic cells which now constitutes a ganglion, contains a mass of fiber-like 'Punkt Substance' of the Germans, the nerve fibers. The nerve fibers are of two kinds, the longitudinal and the transverse (fig. 120), the former running parallel to the long axis of the body, connecting with those of adjoining ganglia, while the latter run at right angles to the longitudinal fibers.

The formation of nerve fibers takes place much the same as was described for Xiphidium by Wheeler ('93) and for the honeybee by Nelson ('15), namely, by the elongation and subsequent transformation of the cytoplasm and the disappearance of the nucleus of the ganglioblasts (fig. 116).

The neurogenesis described above agrees, in general, with the results obtained by Wheeler in Doryphora and Blatta ('89) and for Xiphidium ('93), Heymons for Forficula ('59), Graber for Melolontha ('90), Lecaillon for Chrysomelidae ('90), Nelson for Aphis mellifera ('15), and several other investigators in the case of many other insects.

As to the number of neuroblasts which produce the ganglioblasts in each half of a body segment, no two investigators

exactly agree. Wheeler, for example, found four in Xiphidium, while Nelson stated that in the honey-bee the number of these cells usually varied from three to six. In coccids there are six on each side of the median groove.

Again, opinions are at variance as to whether the ganglioblasts become directly converted into the nerve fiber or into their daughter cells which constitute the nerve cells of the larva. Wheeler ('89) for Doryphora and Nelson ('15) for the honey-bee claim that all the ganglioblasts undergo at least one division before they become nerve-cells, while Wheeler for Forficula ('95), Lecaillon ('98) and Hirscher for Chysomelidae, and Esscherisch ('02) for Musca maintain that the ganglioblasts do not divide. In the coccids, as stated, the ganglioblasts do not undergo any division, but, later, some of them are transformed into nerve fibers.

The neurogenesis in the case of the brain is exactly the same as in the ventral nerve cord. The differentiation of the ammion, mesoderm, and ectoderm is shown in figure 105. The ectoderm cells soon give rise to the neuroblasts and thus are differentiated into the dermal and neural layers. The segmentation of the brain into three regions, corresponding in number to the future brain segments, is also clearly noticeable in this figure.

Figure 110 is a corresponding longitudinal section of a somewhat older embryo. There are about five large spherical cells or neuroblasts along the periphery of each of the brain segments, as was the case with the formation of the ventral nerve cord.

In Coccids, as in other insects, there is originally one ganglion in each of the body segments, making three in the brain, three in the oral region, three in the thorax, and ten in the abdomen, or nineteen in all. This number of ganglia is best seen in the specimens shortly after the completion of the revolution (figs. 53 and 118). Shortly after the union of the stomadeum invagination with the entoderm or the midgut, all of the abdominal ganglia, except the first, disappear, leaving only the longitudinal nerve fibers behind. These slender longitudinal nerve fibers, it may be added, run out from the posterior margin of the only surviving abdominal ganglia and innervate the abdominal organs, such, for example, as the ovaries, midgut, etc. (fig. 132).

A special effort was made to locate the presence of the second antennal appendages and their ganglia, which were found and described by Riley and others in Blatta germanica. My figure 114, which corresponds approximately to Riley's figure 5, shows neither appendages nor ganglia. Throughout the embryonic period there was no indication of the formation of the second antennal appendages or the ganglia corresponding to them.

The apparently later increase in size of the brain has already been mentioned in connection with the formation of the external form of the body. The statement, however, does not mean that neurogenesis in this region lags behind that in the ventral nerve cord. In fact, neurogenesis in the former occurs and progresses just as early as in the second maxillary segment, and, consequently, is ahead of that in the thoracic segment. In this regard, then, coccids seem to differ from aphids, in which, according to Will ('80), the brain arises independently from the ventral nerve cord, as in the case of the annelids.

Again, as my figures 62 and 67 show, the brain in coccids is formed within and not beyond the rim of the blastopore, as Will found in agamic aphids.

8. THE INTRACELLULAR SYMBIOSIS

Intracellular organisms in the eggs of scale insects have been described under several different names, such as: 'Pseudonavicellae' (Leuckart), 'secundaren Yolk' (Mecznikow), "Highly refractory bodies with specific gravity higher than water and may represent spermatozoon" (Putnam), and 'Mycetomia' (Emeis). The only investigator who denied the presence of such organisms is Witlaczil, while Johnston, Child, and other students of the internal anatomy of coccids failed to mention either their presence or absence.

Mention has already been made of the presence of an organism in the mealy bug, in the cottony cushion scale, and in Lecaniodiaspis prunosa. These bodies present an appearance of true cells at certain times in their history, but they more often stain heavily and hence look like granules. Whenever they assume a true cellular form, a cell membrane, chromatic granules, and cytoplasm

can be made out. That they are really a kind of parasitic organism may be proved by the fact, according to Blüchner, that they were successfully raised in culture media by an Italian investigator. As I am not familiar with the systematic position of the different kinds of organisms found in the eggs of the several species of coccids, I describe them under the somewhat indefinite yet suggestive terms of 'symbiotic organisms,' 'parasitic organisms' and often as 'polar colony of organisms,' or simply as 'parasites.'

The manner in which the eggs become infected by the organisms may be observed by examination of older ovarian eggs, such as is shown in figure 25. Here a few dark granular bodies occur in the follicular epithelial cells situated above the constriction between the nurse cells and egg chamber and also in the egg proper. These dark staining bodies lie at first in the cytoplasm and not in the nucleus of the follicular cells. Since, as illustrated, these parasites are found, not only in the epithelial cells, but also in the lumen between the epithelial layer and nurse cells as well as within the egg, it is clear that they are carried into the egg protoplasm mostly by the flow of the nurse stream. Soon, however, the chorion is formed around the cytoplasm of the egg, preventing a further immigration of the symbiotic organisms into the egg. In many cases I have noticed the organisms between the ovarian epithelial layer and the chorionic membrane, long after the completion of the chorion around the egg. But no case has been observed where these bodies actually penetrated through the chorion into the egg.

The condition of the nurse chamber and also that of a portion of a colony of the symbiotic organisms that almost envelops the ovary at this period is shown in figure 40. A careful examination of the specimen brings out the fact that these organisms, so abundant in the cysts adjoining the ovaries, become literally squeezed out of the walls of the cyst into the space between the ovary and the symbiotic organisms. In short, all series of transitional stages between the liberation of the parasites and their entrance into the epithelial cells, and final location at the posterior end of the egg can be found in a few sections of a single female.

The parasites, thus finally collected into a single mass, remain at this position throughout the embryonic as well as the larval and adult life. This place, it should be mentioned, corresponds to the third to fourth abdominal segments of the future embryo and adult.

After the completion of the blastoderm and the subsequent invagination of the germ band (which occurs at about the posterior end of the egg), the germ cells, the first differentiated cells, migrate toward, and some of them actually become imbedded in this colony of symbiotic organisms. Of the migratory cells, those which become imbedded in the mass of parasitic organisms are transformed into the secondary yolk cells of Will ('88). The nuclei of the secondary yolk cells remain almost unaffected for a long time, even after hatching. Again, some of the cells surrounding the colony of organisms transform into epithelial cells, while a majority of them divide, multiply and finally become the definitive germ cells.

As previously stated, the invaginating germ band gradually increases in length until the caudal region becomes actually curled over the thoracic region, but the colony of symbiotic organisms remains almost stationary at the place where it was first located soon after the entrance into the egg. This fixed place, as previously stated, corresponds approximately with the third and fourth abdominal segments of the future embryo and also of the larva and adult.

From this time on, the spherical colony of organisms, consisting of about eight compartments of spores, becomes spread out over the rudiments of the ovaries. The organisms gradually increase in number after hatching until they fill the greater portion of the coelomic area of the adult females.

These organisms are also found in enormous numbers, similarly located, in the larval as well as the adult males. From the fact that these organisms remain throughout the life history of the scale insects, I strongly believe that they are not sperm cells as Putnam ('88) surmises. It also clearly shows that they do not function as 'Keimbahn determinants' as Mecznikow doubted in the case of Psylla.

In the case of Icerya, the symbiotic organisms appear in a somewhat different manner, especially as regards the place where they first enter. A brief account of the mode and place of entrance of the Iceryian form, therefore, may not be out of place.

In the cottony cushion scale, the symbiotic organisms first appear, not around the anterior, but at the posterior end of the egg. The method of entrance is, however, essentially the same as in the case of Pseudococcus and Lecaniodiaspis already mentioned. After their liberation from the cytes, the rod-like organisms migrate through the epithelial layer surrounding the posterior end of the egg. In figure 35 several stages in the migration of these rod-like organisms into the egg are shown. These, like those of Pseudococcus, stain exceedingly darkly with iron alum haematoxylin or any other nuclear stain, and consequently their finer structure is difficult to make out. Nevertheless, more favorable specimens show at least three regions in each of them. These are the outer somewhat lightly staining portion, probably representing cytoplasm; the inner more densely staining region, resembling the nuclear region; and a central denser region, probably representing chromatic matter. Since, as stated, the symbiotic organisms, in the case of the cottony cushion scale, become localized near the posterior end of the egg, where the invagination of the germ band occurs soon after the final settlement of the organisms, the germ cells come into contact with the parasites without migrating far. But, since now the germ cells and the symbiotic organisms are situated at the point of invagination, they both become actually pushed into the egg further and further, as the invaginating germ band elongates (figs. 62 to 67), until they become similarly located as in the mealy bug and Lecaniodiaspis. As soon as they reach this point, a short distance from the anterior pole of the egg, they become stationary and do not accompany a further extension of the caudal portion of the embryo. This fixed point corresponds, as in the case of other scale insects, with the third and fourth abdominal segments of the embryo, larva, and adult. The history of these organisms from this time on is an exact repetition of what has been described for the other two species.

The symbiotic organisms in Homoptera were first discovered in the pathogenetic embryo of aphids by Huxley ('58). Mecznikow ('66) detected a similar substance not only in the embryos of aphids, but also in those of coccids and Psylla. This writer thought his 'secundaren Dotter' to be characteristic of three families of Homoptera, namely, Aphididae, Coccidae, and Psyllidae. Witlaczil ('84) and Will ('88) confirmed the presence of 'pseudovitelli' so far as the parthenogenetically developing embryos of aphids are concerned. The occurrence of similar granules in the winter or sexual eggs of Aphids was first reported by Balbiani ('74) and was later confirmed by the researches of Tannreuther ('07) and Webster and Phillips ('12). All of these writers, however, did not consider these as living organisms. Huxley ('58) was also the first who described the origin of the 'pseudovitellus.' He states that the pseudova (parthenogenetic eggs) of Aphids are eventually converted into cellular germs, apparently by the same process as that by which an ovum is converted into an embryo. "In these germs," he claims, "the central part becomes a granular pseudovitellus, the peripheral a blastoderm; the rudiments of the different organs next appear, and the germ becomes surrounded by a pseudovitelline membrane. Eventually," he supposed, "the pseudovitellus becomes the corpus adiposum."

Mecznikow ('66), however, observed the origin of the pseudovitellus in aphids. He stated that the so-called secondary yolk comes from the follicular cells situated at the posterior end of the egg. This discovery was later confirmed by Witlaczil ('84) and Will ('88).

Emeis ('15) recently found a case of symbiosis in the eggs of Coccids. These symbiotic Mycetomia were first found in a certain epithelial cell near the nurse cells. Later, according to him, they migrated into the protoplasmic portion of the egg. Their subsequent history has, however, not been studied.

Inclusions other than the secondary yolk, but somewhat related to this substance, were recorded for insects belonging to several different orders. Blochman ('87) noticed a group of bacteria-like organisms which he called bacterial 'Stabchen' in the eggs

of certain Orthoptera. He did not state how the eggs were infected, but thought it was a case of symbiosis. The occurrence of non-living substances has also been recorded in the eggs of several Diptera and Coleoptera. These so-called Keimbahn-determinants are located at the posterior end of the egg, as is the case with the secondary yolk or parasites in Hemiptera, but are known by several names. Thus Ritter ('11) gave the name of 'Keimwulst' for the Keimbahn determinants of Chironomus, while Hasper ('11) termed them 'Keimbahnplasma.' A similar substance is described as 'Dotterplatte' in Calliphora (Kahle, '08), but is called 'polares plasma' in Miaster (Hegner, '12). Recently Hegner ('09) applied the term 'pole disc' to a related substance in the eggs of chrysomelid beetles. The same nomenclature was adopted by Wieman ('10) two years later for an allied species.

The origin of the Keimbahn determinants in these insects is still unknown. Hegner ('08, '10) states that he has attempted to trace their origin, but failed to arrive at an exact source in the case of the eggs of Chrysomelids (Hegner, '15). It was thought, however, that as in the Hymenoptera chromatin granules might be cast out of the nuclei of the oocytes, and that these granules might gather at the posterior end to form the pole disc. It was also suggested that chromatin granules from the nurse cell nuclei might make their way into the oocyte and later become the granules of the pole disc. It should not be forgotten, moreover, that these granules stain like chromatin. In fact, they are non-living substance and therefore cannot be homologous with the simbiotic organisms found in Homoptera. There is, however, an analogy in the relative position of these two kinds of polar inclusions, namely, the symbiotic organisms found in Homoptera and the polar granules of eggs of Diptera and Coleoptera. The germ cells of these insects become lodged in the polar granules or symbiotic organisms early in the embryonic period.

As to the function of the symbiotic organisms found in aphids and coccids, very little can be said. As already stated, the germ cells of coccids, before their migration into the colony of the symbiotic organisms, are already differentiated. This excludes

the possibility of the function of the organism as the 'Keimbahm determinant,' as Mecznikow surmised. The fact that these organisms are present throughout the life history of scale insects is in favor of the view just mentioned.

However, I cannot think that the presence of these symbiotic organisms is altogether without significance. The species known to harbor these symbiotic organisms, so far as present researches go, belong without exception to the suborder Homoptera, which are characterized by a long sucking proboscis, very delicate membranous wings, and thin dorsal body wall. Coccids may remain more or less stationary, feeding in the same place, for a long period of time. It is clear, therefore that such Homoptera as the scale insects and the aphids are apt to be exposed to changes in the external conditions because they cannot change places as easily as other insects.

It may be said that the part most susceptible to the environmental change is the sex cells. As a rule, the sex cells of the Homoptera, unlike those of the Coleoptera, in which the hard elytra cover the dorsal surface of the body, lie beneath a thin, almost unprotected dorsal body wall. Unless some special means be provided for their protection, the sex cells would be in danger of injury. In free moving Homoptera, such as Jassids, Membracids, Flugorids, Cercopids, the means of avoiding injury to the germ cells are found in quick movements by which these insects are able to take refuge by either dropping to a more favorable place or running behind the trunk or leaves of the host plant. In the Coccids, Aphids, and Psyllids this change of place cannot be effectively made. The presence of a mass of highly refractory organisms that always surrounds the germ cells in these three otherwise helpless insects strongly suggests a significance—the protection of germ cells against injury and sudden change in environmental conditions, such as rain, snow, extreme heat, cold, etc.

9. THE ORIGIN OF THE GERM CELLS

In coccids the germ cells first become noticeable simultaneously with the invagination of the germ band at the posterior end of the egg. They are characterized by their large size, oval shape, and the clearer nuclear appearance as compared with the other cells, and, above all, by the feeble staining reaction. Increasing in numbers by mitotic division, they remain for a time at the point where the invagination of the germ band occurs. The earliest stage in the history of these cells which has been observed is shown in figure 81.

At the time the invagination becomes clearly visible, the germ cells begin to migrate, one after another, toward the anterior end of the egg where the colony of symbiotic organisms is located. As soon, however, as they reach the mass of organisms, some of them become actually imbedded between the spores of the organisms, others elongate to form a sheath around it, while the remaining cells aggregate superficially around the colony of organisms (figs. 90, 92, and 133). Those cells that become imbedded in or encircle the organisms remain in that condition throughout the rest of the embryonic and larval life and constitute the so-called secondary yolk cells of Will ('88) (fig. 90).

As already mentioned, the elongation of the embryo continues beyond the anterior end of the egg so that the posterior portion gradually curls over the thoracic region, but the germ cells and the colony of the symbiotic organisms remain stationary. The region where these cells, for the second time, become localized, corresponds with the third and the fourth abdominal segments of the larvae as well as of adult female. With the segmentation of the abdominal region of the embryo, the germ cells become divided into the left and right halves.

With the revolution and subsequent shortening of the embryo an invagination appears at the ventral surface of the ninth abdominal segment. The germ cells, which have for a long time remained stationary around the symbiotic organisms, then migrate toward and finally settle at the apex of the vaginal invagination (fig. 119).

Following the closure of the dorsal wall of the embryo along the dorsal midline by the lateral growth of the ventral plate, and with the further ingrowth of the vaginal invagination, the germ cells are carried further cephalad and thus again come into contact with the colony of the symbiotic organisms. But this time they collect beneath, instead of encircling the organisms, as was the case at first. Throughout the larval and adult stages the germ cells and the colony of symbiotic organisms retain their relative positions. The former is always found beneath or ventrad of the latter as though they were under the protection of the latter.

The foregoing account of the history of the germ cells in the mealy bug is essentially applicable to that of Lecaniodiaspis. One of the striking differences is that in the case of Lecaniodiaspis the germ cells begin to migrate at a much later period than in Pseudococcus macdanieli. The number of germ cells that migrate toward the symbiotic organisms in the case of Lecaniodiaspis is very much smaller than in the case of Pseudococcus (fig. 89).

Inasmuch as the origin and subsequent history of the germ cells in the cottony cushion scale is somewhat different from what has already been given for the mealy bug and Lecaniodiaspis, a short account for this species may be added here.

In the cottony cushion scale the germ cells become noticeable at the posterior pole of the egg just as in the mealy bug. They are larger, clearer, and their nuclei stain much more feebly than those of the other cells around them. Although there is no doubt that they, like those of the mealy bug and Lecaniodiaspis, are able to migrate, the movement is not well marked on account of the comparatively short distance that separates the blastodermic layer from which the germ cells are derived and the colony of symbiotic organisms. The germ cells of the cottony cushion scale come into contact with the organisms earlier than in the other two species, since the migration is a short one. This fact of the early association of the germ cells with the symbiotic organisms, and also the posterior position of the latter, led Mecznikow ('66) to a statement that the symbiotic organisms

which he called the pseudovitellus may act as the Keimbahn determinant in coccids and especially in Psylla.

Following this stage, the germ cells become pushed anteriorly by the invagination of the ventral plate. When, however, the colony of symbiotic organism becomes stationary near the posterior end of the egg, the germ cells become also fixed, so to speak, at the place which corresponds approximately to the third and fourth abdominal segments. When the abdomen begins to show segmentation, the mass of germ cells becomes detached from the colony of parasitic organisms and aggregates to form left and right syncytia, the miniature yet definitive ovaries.

Thus the chief difference between the germ cells of the mealy bug and of Icerya is that, in the former, the germ cells early migrate to the anterior colony of symbiotic organisms on their own accord, whereas in the latter they move anteriorly, not by their own effort, but by the ingrowth of the germ band.

The migration of the germ cells, singly or as a group, as I have observed in coccids, is by no means peculiar to this family of insects. Such an instance has already been described for the embryo of the potato beetle, Leptinotarsa decemlineata, by Hegner ('14), who found that the germ cells which apparently rest in the amniotic cavity migrated later on into the embryo through a sort of canal at the bottom of a groove in the germ band.

In the two families of Homoptera, Aphididae and Psyllidae, which are closely related to Coccidae in form, life history, and habits, the primitive germ cells were observed, just as in the case of the coccids above mentioned, early in the embryonic development. Mecznikow ('66) described the primitive germ cells of parthenogenetically developing embryos of Aphis (Macrosiphum) rosae before the appearance of mesoderm cells. Eighteen years later, Witlaczil ('84) not only confirmed Mecznikow's observation, but was also able to see a single large clear cell, the primitive germ cell, near the blastopore.

The primitive germ glands of chrysomelid beetles have also been found to appear at about the same stage of development as in the case of aphids. In Clytra laeviuscula, Gastrophysa raphani, Chrysomela menthantri, Lina populi, and Lina tremulae,

studied by Lecaillon ('98), no differentiation has been observed among the cleavage cells until their migration to the periphery, when they become the blastoderm cells. Although the point at which the first cleavage cell reaches the periphery differs with the species, yet in all species which have been investigated so far a complete layer of blastodermic cells forms around the periphery of the egg. Those cleavage cells that eventually come to lie around the posterior pole of the egg where the polar granules are located, become larger and clearer than their neighboring cells. They then become disconnected.

Wheeler ('89) was not so fortunate as to observe such an early segregation of the primitive germ glands in Leptinotarsa (Doryphora) decemlineata. According to him, these cells arise as two elongated thickenings of splanchnic mesoderm. Saling's observation ('07) on the development of genital cells in Tenebrio molitor is in accord with those of Heider and Wheeler in Hydrophilus and Leptinotarsa. The germ sells of Hydrophilus piceus also arise at a much later period of development. According to Heider ('89), the germ glands of this beetle are derived from the inner wall of the primitive abdominal segments on either side of the body. At first they are indistinguishable from the neighboring mesodermal cells from which they originate, but soon they grow in size, and their nuclei become clearer. In the following Orthoptera the germ cells have been observed to develop in such a manner and at about the same period of embryonic development as in Hydrophilus:

Oecanthus niveus, Ayers ('83).

Blatta germanica, Heymons ('90–'91); Wheeler ('99).

Periplaneta orientalis, Nusbaum ('66).

Xiphidium, Wheeler ('93).

The sex cells of the Hymenoptera have been derived from mesoderm cells in much the same fashion as in the Orthoptera. This statement agrees with the researches of Grassi ('84) for Apis, of Carriere and Burger ('97) for the mason-bee (Chalicodoma), of Petrunkewitsh and of Nelson ('15) for the female honey-bee.

Several cases of germ-cell segregation during an early cleavage stage have been recorded in Diptera. As early as 1862, Robin described what seemed to be the primordial germ glands of Tipulides culiciformis. According to this investigator, four to eight buds were seen at the posterior end of the egg. A similar observation was made by Weismann a year later in the egg of Chironomus. On account of their position Weismann called them 'die Polzellen.' Both Robin ('62) and Weismann ('63) failed to trace these cells up to the formation of definite organs. Metznikow ('65, '66) and Leuckart ('65), however, found that the 'Polzellen' of Weismann migrate into the body cavity of the embryo and become the sex cells in Simula, Chironomus, Culex, and Miastor. In the following Diptera, studied by several investigators since then, germ cells first become differentiated before the completion of the blastoderm:

Chironomus, Grimm ('70).
Chironomus, Weismann ('82).
Chironomus, Balbiani ("82–85).
Chironomus, Jaworowski ('82).
Musca, Kowalevsky ('86).
Miastor, Kahle ('88).
Miastor, Voeltzkow ('89).
Calliphora, Lucilia, Graber ('89).
Chironomus, Ritter ('90).
Musca, Escherich ('00).
Calliphora, Noack ('01).
Chironomus, Hasper ('11).
Miastor, Hegner ('13–'14).

Meyer ('49) could not find the genital organs of Liparia auriflua, one of the Lepidoptera, until the caterpillars were over three weeks old, while Balnini ('69–'72) found them in the embryo of Tinea crinella at about the time the segmented appendages make their appearance.

Woodworth ('89) and Schwangart ('05), however, found a comparatively early differentiation of germ cells in certain butterflies. They found a thickening of the blastoderm near the posterior end of the egg, the inner cells of which differentiate

into germ cells. Later these migrate singly into the fourth to the eighth abdominal segment and there become the forerunner of the ovarian cells.

The primitive germ cells in Forficula auricularis were also found by Heymons ('95) at about the same stage as in the case of the butterflies mentioned above.

Heymons ('96), who could not find the genital organs in the embryos of certain dragonflies and May-flies, is of the opinion that in the Ephemeridae and Odonata the first genital rudiments seem to appear during the larval life. This is, indeed, a case of the latest segregation of the germ cells so far recorded among the insects.

From the foregoing brief survey of the germ-cell formation in insects, it becomes clear that a considerable variation is evident in the manner and also in the time of their differentiation. This variation is not a continuous one, but is such that the insects, whose germ cell formation so far has been studied, can be grouped in the following three categories:

1. Cleavage differentiation: Diptera, possibly Hymenoptera.
2. Blastodermic differentiation: Aphids, Chrysomelids, Lepidoptera and Coccids.
3. Mesodermic differentiation: Orthoptera, Aptera, Neuroptera, Hymenoptera, parasitic forms, Ephemeridae, Odonata, Dermaptera and probably Heteroptera.

It is interesting to note that in all insects, the germ cells of which have not been observed prior to the formation of mesoderm, no special posterior inclusions have been found. Conversely, in all the eggs having a certain kind of polar inclusion, whether it be of nurse cell, epithelial cell or of nucleolar origin, the earliest segregation of the germ cell has been recorded. Nevertheless, it must be stated that the differentiation of the germ cells as such cannot solely be accredited to the so-called 'Keimbahn determinants' of Hegner. The germ cells of the Chrysomelid beetles that possess the polar granules, appear at about the same time as those of aphids and scale insects where there are no such granules, but parasitic organisms. Such a striking case is that of Euvanessa, where there is no possible inclusion of any sort

comparable to those of Coleoptera and Diptera, yet, even in this case, the germ cells arise just about the same time as those of Coleoptera. The facts just mentioned, that the germ cells of coccids, especially of Pseudococcus, become distinguishable as such long before their approach to the colony of parasites, indicate that the germ cells are by some chemotaxic action attracted to the inclusion and do not become germ cells because they come in contact with the latter.

10. THE FORMATION OF THE DIGESTIVE TRACT

The digestive tract of the embryo of the scale insects consists, as in other insects, of the fore-, mid-, and hind-gut. The proctodæum and stomodæum in the scale insects are formed in exactly the same manner as in other insects. The stomodæum grows from its ventral opening, the mouth, dorsally, traversing the future nervous rudiment almost perpendicularly, until it meets the long axis of the egg, and then it turns posteriorly and meets with the midgut rudiment. The posterior (proctodæum) invagination, on the other hand, takes place on the tenth abdominal segment, the segment which, at the time when the anus is externally visible, still lies curled over the thoracic segment (fig. 50). From this time on until the embryo completes its revolution, the posterior invagination proceeds very slowly, as a comparative study of the figures 126 and 127 will show. However, the rate of invagination after the revolution is exceedingly rapid.

The midgut of Pseudococcus macdanieli Hollinger (MS) arises from the entodermal cells, the origin of which has already been described.

The condition of the entodermal cells, at the time the thoracic appendages begin to have two segments, is shown in figures 93 to 96. These represent consecutive longitudinal sections of the same embryo. The purpose of giving these serial sections is to show that the cells forming the entoderm are not in any way connected with the cells surrounding the colony of symbiotic organisms; that they are an altogether different kind of cells; that they are clearly separated from the mesodermal layer, and that they are also distinct from the ectodermal cells. The

entodermal cells, as illustrated in these figures, constitute two parallel layers extending from the caudal extremity, where the three germ layers arise, cephalad as far as the sixth or seventh abdominal segment. The cells of the ectodermal layer at this stage are ellipsoidal, and their nuclei stain exceedingly dark with iron-alum haematoxylin, so they can be distinguished easily from the spherical cells lying below them. The spherical cells, which form a layer closely opposed to the ectoderm, are larger, with richer nuclear contents than the cells of the ectoderm. Thus it is clear that there is no possibility of confusing the entoderm cells with the cells of the two other germ layers.

The germ cells situated around the colony of symbiotic organisms, however, resemble the entoderm cells in many respects. The common origin of the germ cells and entoderm cells, however, can be disregarded by the facts that they originate at the two different periods in the development of the embryo and that, at any time during their respective histories, the former never migrate caudad beyond the third and the latter cephalad beyond the seventh abdominal segments.

Figures 126 and 128 represent two longitudinal sections of different embryos, similar to those in figures 48 and 51. In both cases the entodermal cells are much compacted near the posterior end of the embryo. A crescentic groove is shown in figure 126. This compacted condition of the entoderm and the appearance of a clear groove within the entoderm cells are both due, not to the actual massing or proliferation of cells as may be surmised, but to the proctodaeal invagination which causes the elevation of the posterior portion of the entodermal layer.

Following these stages, the entoderm cells multiply rapidly and form a coiled tube (fig. 129), so that a transverse section through the fourth abdominal segment passes through the entoderm three times as shown in figure 113. The midgut tube is pushed still further in toward the cephalic end of the embryo by further ingrowth of the proctodaeum until its anterior end meets and unites with the posterior elongation of the stomodaeum, the history of which has already been described. The condition of the alimentary canal shortly after the union of the stomodaeum with the midgut is shown in figure 14. During the stages

that follow the wall of the proctodaeum gradually becomes thin and membranous, while the midgut increases in size (fig. 130). After hatching, however, the cephalic portion of the midgut becomes enlarged and finally assumes a shape very much resembling the human stomach, while the stomodaeum remains a slender tube throughout (figs. 56 and 132). A greater portion of the midgut of certain coccids, especially that of Aspidiotus nerii, was thought by Mecznikow to be derived from the elongation of the proctodaeal invagination, because, as he stated, the alimentary canal of coccids is very short. In the case of Icerya it was a very difficult matter to distinguish the rudiments of the midgut in the early stages, and consequently I might have also arrived at the same conclusion as Mecznikow did, had I not also studied its development in the mealy bug.

In all insects both the fore- and hindgut are derived from ectoderm, according to different investigators, with the exception of Diptera, in which, according to Voeltzkow ('88) and Graber, they are of mesodermal origin.

In the aphids, which are closely related to the coccids, the alimentary system is, however, derived from a different source, according to investigators. The fore and hindguts are here also formed from the ectodermal invaginations. The midgut is claimed by Will ('88) to come from yolk cells, but according to Witlaczil ('84) the same organ is formed from the proctodaeal and stomodaeal invaginations and therefore is strictly ectodermal in origin.

In the majority of insects, however, the midgut is formed from the anterior and posterior rudiments. These rudiments were frequently spoken of as entoderm cells, but more often claimed to be ectodermal in nature. The supposed entoderm cells in the case of the honey-bee are, according to Nelson's research, derived from anterior and posterior portions of the blastodermic tube which is in direct continuation with the side plate or ectoderm.

Thus, the point of interest in the formation of the midgut in coccids is this: the midgut is entirely derived from its rudiments, the entoderm cells, situated at the posterior end of the embryo. This is, so far as my knowledge goes, a new type of the midgut formation, never recorded in the case of the class Insecta.

11. SUMMARY

1. The three ovarian elements, namely, the nurse cells, the egg cell, and the follicular epithelial cells, are derived from the primordial germ cells. All germ cells are exactly alike during the oogonial period. The first differentiation begins after the next to the last oogonial division. Few peripheral cells undergo the last oogonial mitosis and enter into the so-called growth period of the oocyte of the first order. Their nuclei are, at first, condensed near one pole, but later become thread-like. At this point another change occurs: three or four out of a group of four (Icerya), or five (Pseudococcus and Lecaniodiaspis) oocytes situated peripherally overgrow the single cell situated at the proximal end and secrete a sort of nutritive substance. At the same time their nuclear contents become granular. These are the nurse cells, while the single cell situated at the posterior end becomes the true egg cell. The follicular epithelial cells, which invest the nurse and the egg cells are oogonial cells that happened to be at the proximal part of the group. These epithelial cells multiply, like the somatic cells, by ordinary mitosis and their nuclear contents do not assume the appearance of either a condensed or paired condition.

2. The ovarian egg, at the time of its passage into the oviduct consists of the following parts: a chorion, yolk membrane, cytoplasm, germinal vesicle with its nuclear membrane and contents, and a colony of symbiotic organisms. In addition to these, the eggs of Icerya and Pseudococcus contain fat granules and yolk-like substance.

3. All cleavage cells divide mitotically. No case of amitosis occurs. Some of the cleavage cells later migrate to the peripheral cortical layer and become the so-called blastoderm, while the rest of them remain within the egg and become the so-called yolk cells. The first cleavage spindle lies at right angles to the shorter axis of the egg and the place where the cleavage cell first reaches the cortical layer is at the posterior pole of the egg.

4. The process of ventral plate formation is the so-called invagination type. The invagination occurs at a short distance ventrad to the posterior end of the egg. It is, at first, a shallow

depression, but it gradually elongates to form a long folded tube. Only the dorsal wall of the invagination becomes the embryo proper, while the ventral wall gradually transforms into the amniotic layer.

The second maxillae, first maxillae, thoracic legs, mandibles, antennae, and labrum appear in the order given. As the brain grows enormously in size, the amnion in front of the brain is pushed against the serosa, with which it finally fuses. At this point of fusion a rupture occurs, through which the embryo emerges, first the head, then gradually the rest of the body, and thus it rotates around the transverse axis of the egg, with the result that the poles of the egg and those of the embryo coincide.

A short time before the revolution, a large ventral middle invagination occurs between the maxillae. At the time of the completion of the revolution this invagination forms the ventral cavity. The mandible and the first maxilla each produces, from its distal or pointed end, a long chitinous bristle or oral seta, while the labrum and the second maxilla together form a box-like framework.

5. A short time before the egg passes into the oviduct, a colony of globular (Icerya) or rod-shaped (Lecaniodiaspis) organisms migrate into the egg through the follicular epithelial cells situated at the anterior (Pseudococcus and Lecaniodiaspis) or posterior end of the egg (Icerya). The germ cells, the first definitive cells, migrate toward this colony of organisms and surround it. In the case of Icerya, the germ cells, as well as the colony of the parasitic organisms, are actually pushed forward by the invagination of the germ band to a point a short distance from the anterior end of the egg. Then both the germ cells and colony of the parasitic organisms become stationary and do not accompany the further elongation of the germ band. In the case of Pseudococcus and Lecaniodiaspis, the germ cells which are formed at the posterior end of the egg, voluntarily migrate toward the colony of organisms located near the opposite end of the egg. From the colony of organisms the germ cells later migrate toward the tip of the invagination and form two definitive ovaries.

6. In coccids there is a true gastrula. The entoderm, mesoderm, and ectoderm are all continuous at the tip of the invagination.

7. The rudiments of both the brain and ventral nerve cord are derived from ectodermal cells of the ventral plate. The neuroblasts are formed on each side of the ventral middle line by mitotic division of ectoderm cells. Each neuroblast produces a series of ganglioblasts. The neuroblasts are teloblastic. Ganglioblasts transform into nerve fibers, either longitudinal or transverse.

8. The stomodaeum and proctodaeum are respectively derived from an anterior and a posterior invagination of the ectoderm, while the midgut arises exclusively from the entodermal cells massed at the posterior end of the embryo.

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PLATES

ABBREVIATIONS

a., anus
abd., abdomen
Abdl., abdominal
ag., anal gland
am., amnion
ant., antenna
bld., blastoderm
ch., chorion
cvc., cleavage cell
D., *dc.*, dorsal organ
e., eye
ect., ectoderm
epd., epidermis
epl., epithelial cell
fb., fat cell
gangb., ganglioblast
gc., germ cell
gv., germinal vesicle
ha., hair
hag., hair gland
lb., labium
lbr., labrum
lg1., 1st thoracic leg
lg2., 2nd thoracic leg
lg3., 3rd thoracic leg
m., mouth
md., mandible
meso., mesoderm
midint., rudiments of midgut
mus., muscle
my., symbiotic organisms
Mxl., first maxilla
mx2., second maxilla
nu., nucleus
nc., nurse cell
nb., neuroblast
ng., neurogenic area
nf., nerve fiber
og., oogonium
ocl., ocular nerve
oyt., oocyte
oent., oenocyte
oesp., oesophagus
pb., polar body
proc., proctodaeum
rd., rudiment of abdomen
salg., salivary gland
stm., stomodaeum
sr., serosa
sp., spiracle
ten., tentorium
tr., trachea
v., vagina
vg., vaginal gland
vp., ventral plate
y., yolk
yc., yolk cell
ys?., yolk substance?
1., brain segment 1
2., brain segment 2
3., brain segment 3

PLATE 1

EXPLANATION OF FIGURES

Pseudococcus

1 Ovary of larva at the time of hatching.

2 A portion of sagittal section of the ovary of a larva in early fourth instar.

3 As above. Much later stage.

4 to 15 Stages in differentiation of the three ovarian cellular elements, namely, the nurse cells, egg cell, and follicular epithelial cells.

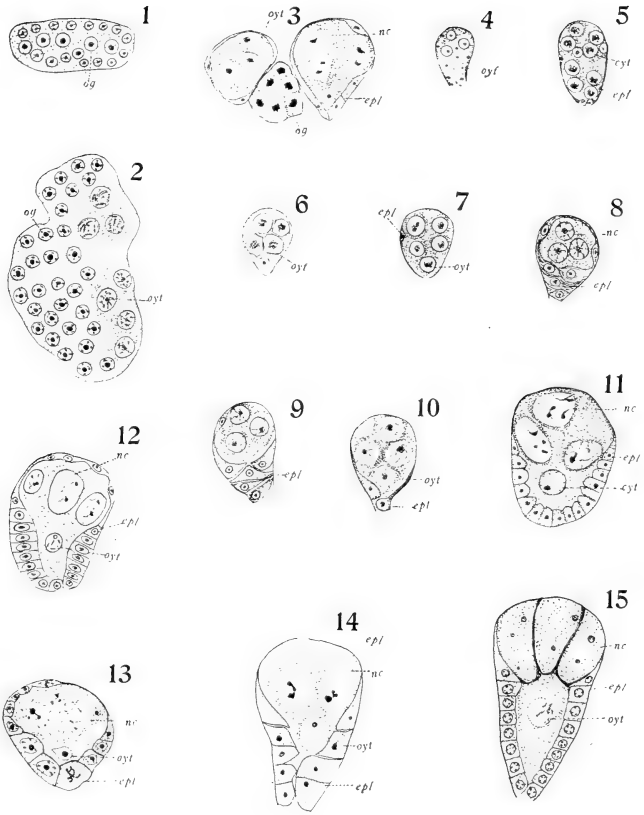
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PLATE 2

EXPLANATION OF FIGURES

Pseudococcus

16 and 17 Stages in differentiation of the three ovarian cellular elements, namely, the nurse cells, egg cell, and follicular epithelial cells.

18 A portion of transverse section through the egg chamber.

19 Transverse section through the nurse chamber like the one represented in figure 15.

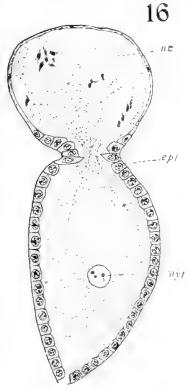
20 Longitudinal section of an ovariole somewhat older than the one represented in figure 17.

21 A portion of transverse section of an ovarian egg.

22 Longitudinal section of an early egg.

23 A portion of longitudinal section through germinal vesicle.

24 Longitudinal section of an egg when the germinal vesicle has reached the periphery of the egg.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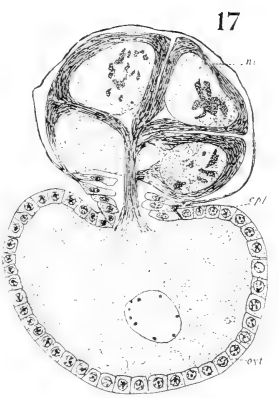
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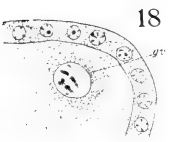
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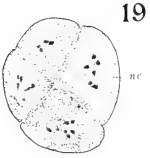
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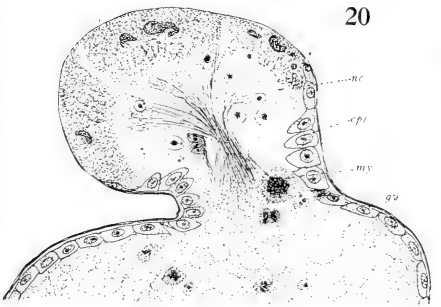
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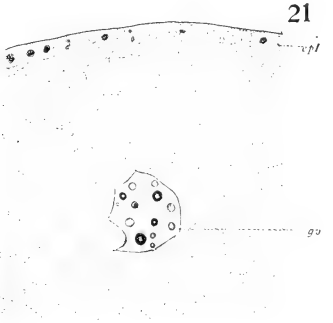
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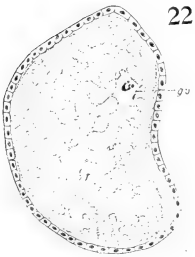
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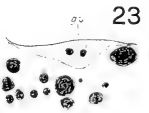
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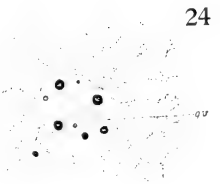
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PLATE 3

EXPLANATION OF FIGURES

Pseudococcus

25 Longitudinal section of an egg somewhat older than the one represented in figure 20.

26 Germinal vesicle in the metaphase of the first maturation division, lateral view.

27 to 28 Longitudinal sections of egg of Lecaniodiaspis a short time before their passage into the oviduct.

29 An oblique longitudinal section of the ovary of an adult Icerya, one hour after copulating.

30 A portion of the ovary of an adult Icerya two days after mating.

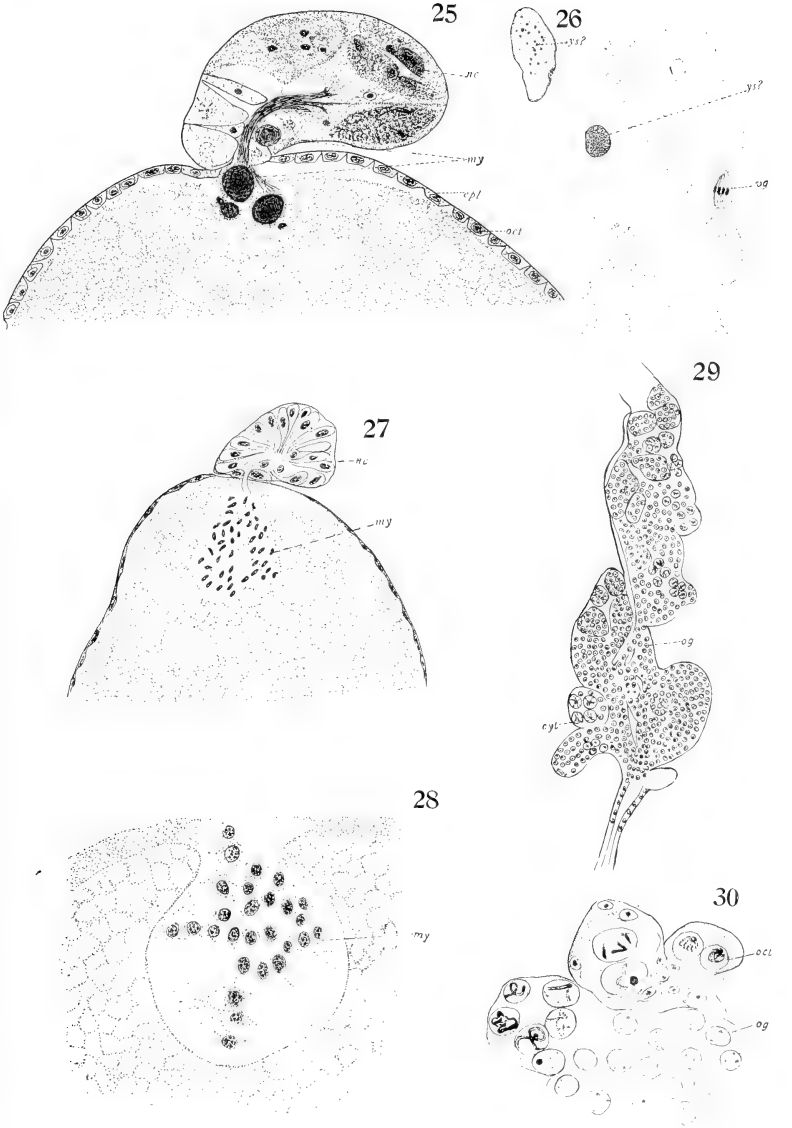
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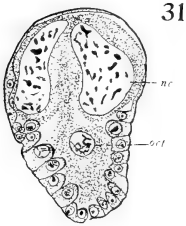
PLATE 4

EXPLANATION OF FIGURES

31, 33, and 34 Stages in growth of the eggs of Icerya.

32 A portion of longitudinal section of an egg of Icerya fixed in boiling Flemming's solution.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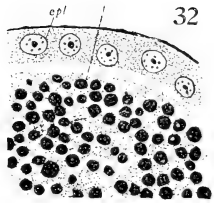
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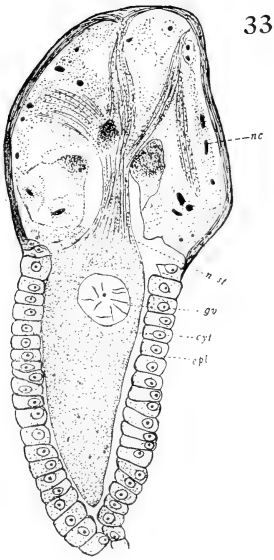
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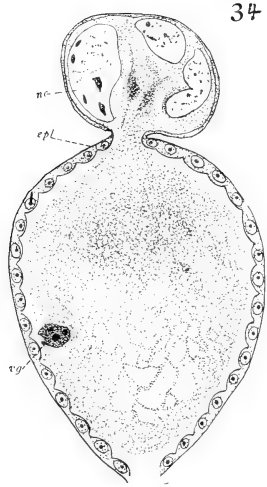
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PLATE 5

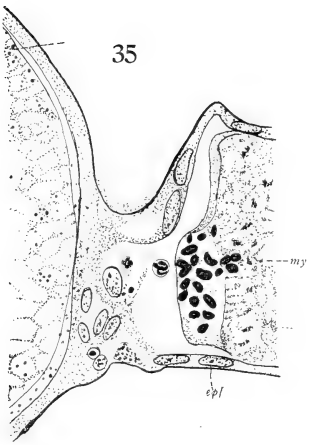
EXPLANATION OF FIGURES

35 A portion of transverse section of the ovary of egg-laying female. Icerya.

36 A portion of transverse section of a Pseudococcus larva in the third instar.

37 Longitudinal section of the egg of Pseudococcus after the polar body formation.

38 The egg of Pseudococcus after the protrusion of the first polar body.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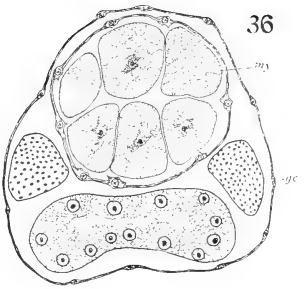
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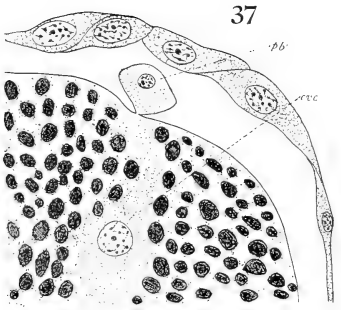
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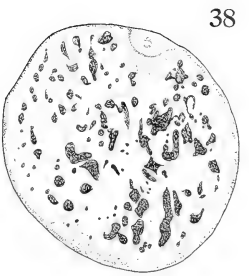
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PLATE 6

EXPLANATION OF FIGURES

39 Longitudinal section of an egg of Pseudococcus, showing only one blastoderm cell and several cleavage cells.

40 Longitudinal section of an egg of Pseudococcus.

41 A portion of figure 43, more enlarged.

42 Transverse section of the egg of Pseudococcus at the time of the completion of the blastoderm.

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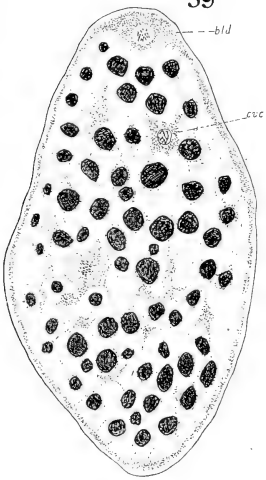

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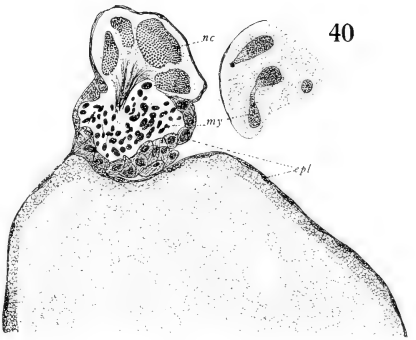

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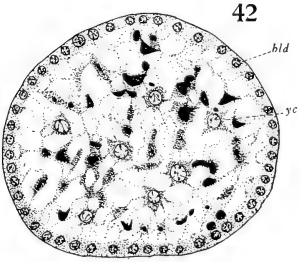


PLATE 7

EXPLANATION OF FIGURES

43 The first cleavage cell of Lecaniodiaspis.

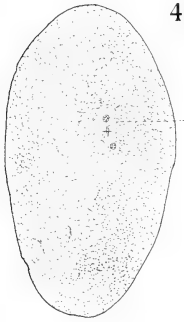
44 Longitudinal section of an egg of Pseudococcus in an early blastoderm stage.

45 to 47 Surface views of embryos.

48 and 49 Surface views of embryos, somewhat older embryos.

50 Lateral view of embryo.

51 Surface view of a somewhat older embryo than the one represented in figure 50.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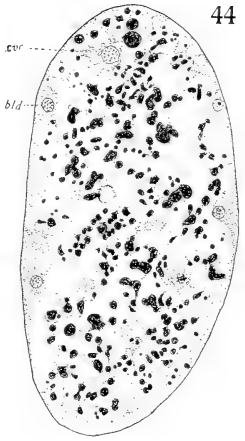
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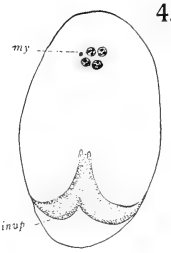
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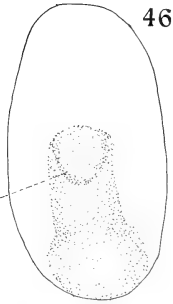
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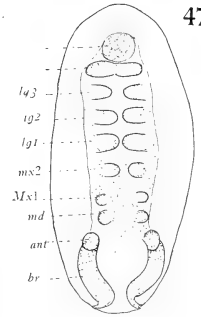
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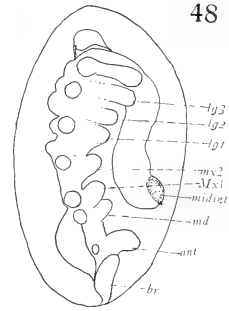
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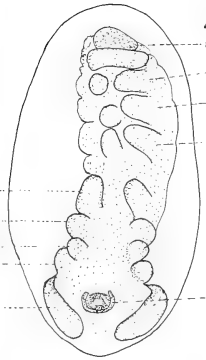
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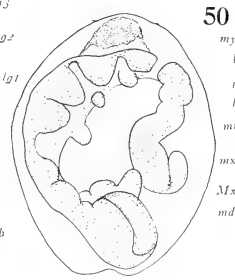

50
my
midint
lg3
lg2
lg1
midint
mx2
Mx1
md

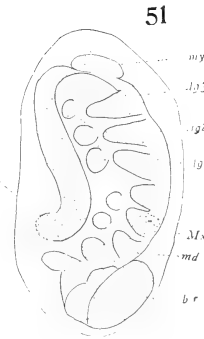

51
my
lg3
lg2
lg1
Mx1
md
br

PLATE 8

EXPLANATION OF FIGURES

Pseudococcus. Toto preparations.

52 Lateral view of an embryo during the process of revolution.

53 Lateral view of an embryo after the completion of revolution.

54 Surface view of an embryo like the one represented in figure 53.

55 Surface view of a much older embryo.

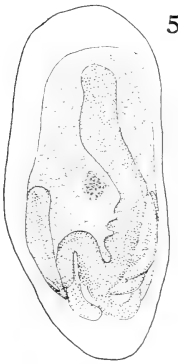
56 Lateral view of still older embryo at the time of the completion of the alimentary canal.

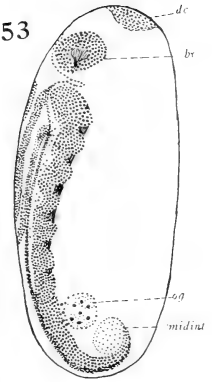
57 A Pseudococcus larva at the time of hatching. Toto preparation.

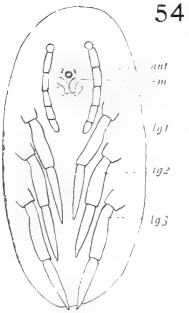
58 An early cleavage stage; toto preparation of an egg of Lecaniodiaspis.

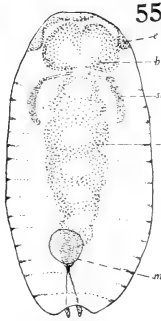
59 Surface view of a Pseudococcus embryo in an early invagination stage.

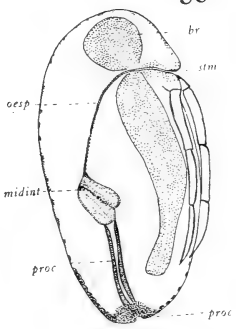
60 Surface view of an Lecaniodiaspis embryo at the time of the appearance of the mouth rudiment.

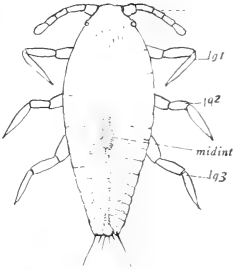

52
my

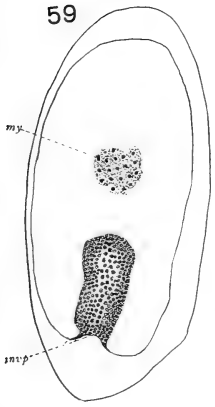

53
dc
br
oq
midint

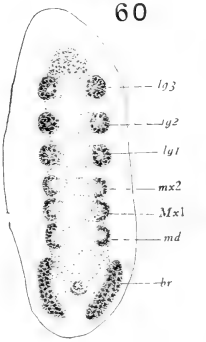

54
ant
m
lg1
lg2
lg3


55
c
br
salg
N
my


56
br
stm
oesp
midint
proc
proc


57
lg1
lg2
midint
lg3


59
my
mvp


60
lg3
lg2
lg1
mx2
Mx1
md
br

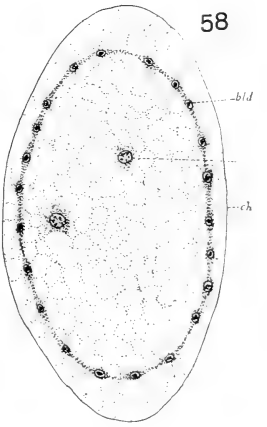

58
bld
ch

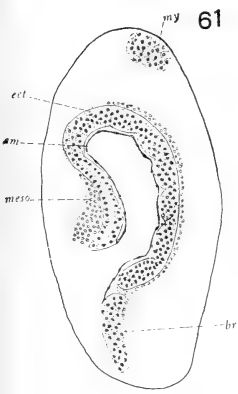
PLATE 9

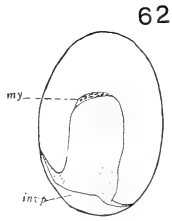
EXPLANATION OF FIGURES

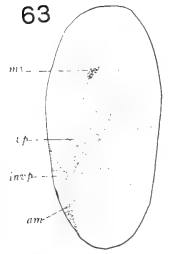
61 Lateral view of an Lecaniodiaspis embryo like the one represented in figure 60.

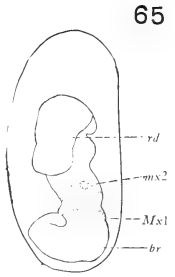
62 to 67 Stages in the growth of the embryo of Icerya.

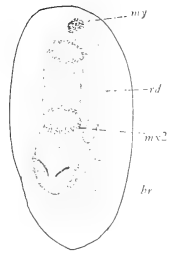
68 and 69 Stages in the development of Iceryan embryo.

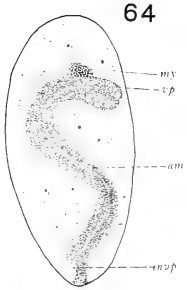

61
my
ect
m
meso
br

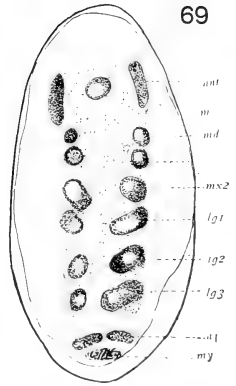

62
my
invp

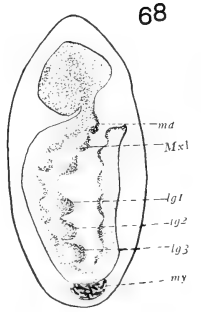

63
mx
vp
invp
am


65
rd
mx2
Mx1
br


66
my
rd
mx2
br


64
my
vp
am
invp


69
ant
m
md
mx2
lg1
lg2
lg3
my


68
md
Mx1
lg1
lg2
lg3
my

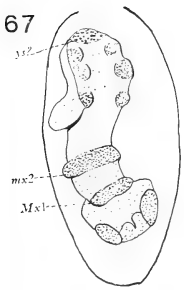

67
ys?
mx2
Mx1

PLATE 10

EXPLANATION OF FIGURES

70 to 75 Stages in the development of Iceryan embryo.

72 Lateral view of an embryo shortly after the completion of revolution.

73 Lateral view of older embryo almost ready to hatch.

74 Embryo at the time of the union of the midgut rudiments with the posterior prolongation of the stomodaeum.

75 Ventral view of a larva eight hours after hatching.

76 Longitudinal section of an Iceryan egg in early cleavage stage.

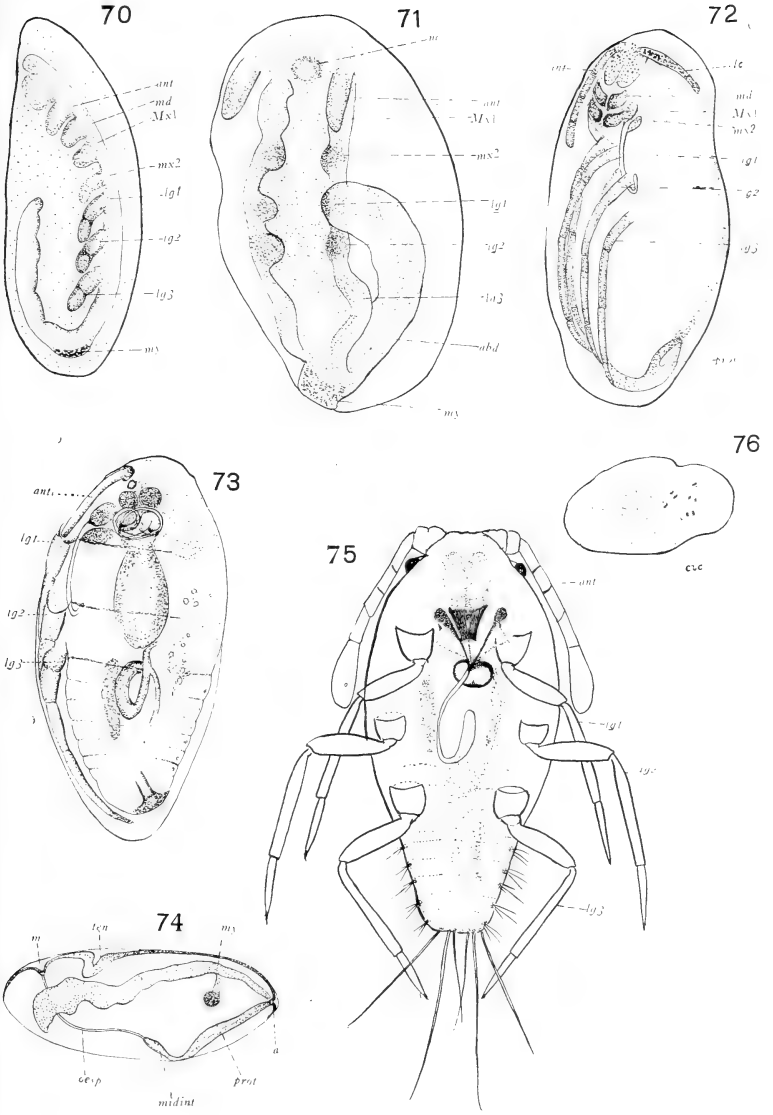

70
ant
md
Mx1
mx2
lg1
lg2
lg3
ms
71
ant
Mx1
mx2
lg1
lg2
lg3
abd
ms
72
lc
md
Mx1
mx2
lg1
lg2
lg3
73
ant
lg1
lg2
lg3
74
m
ten
ms
a
oesp
midint
prot
75
ant
lg1
lg3
76
cuc

PLATE 11

EXPLANATION OF FIGURES

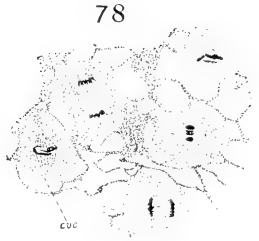
77 Polar view of the metaphase figure, early cleavage cell of Icerya.

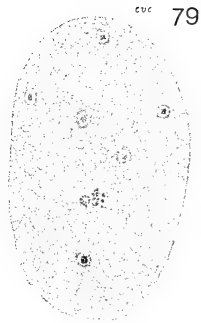
78 A portion of figure 76, much magnified.

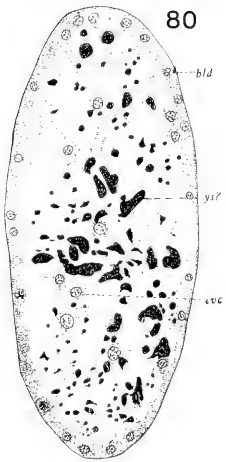
79 Transverse section of an egg of Lecaniodiaspis in early cleavage stage.

81 Posterior half of longitudinal section of the egg a short time after the completion of the blastodermic sac. Pseudococcus.


77
cvc


78
cvc


cvc
79


80
bld
ysf
cvc

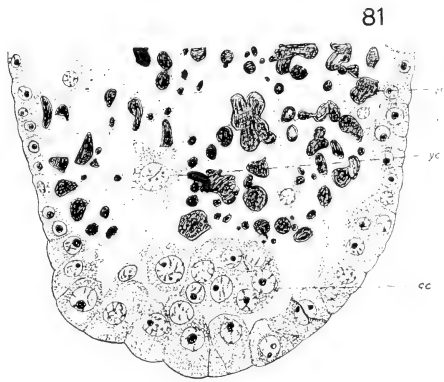

81
yc
cc

PLATE 12

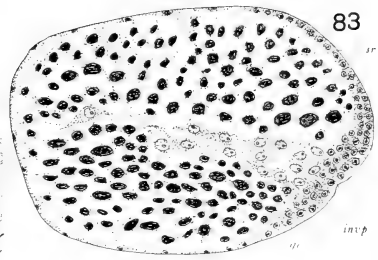
EXPLANATION OF FIGURES

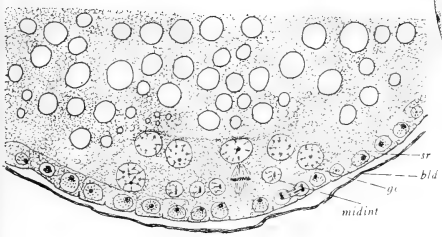
Pseudococcus

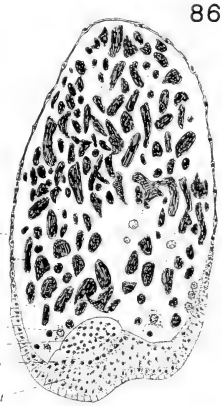
82 and 83 Posterior half of longitudinal sections of the eggs a short time after the completion of the blastodermic sac.

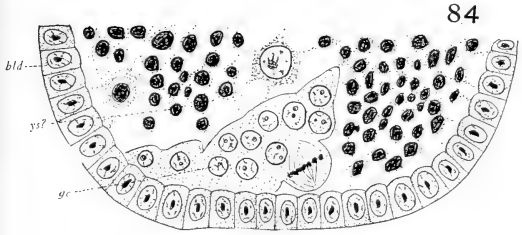
83 Longitudinal (oblique) section of a somewhat older embryo than the one represeented in figure 84.

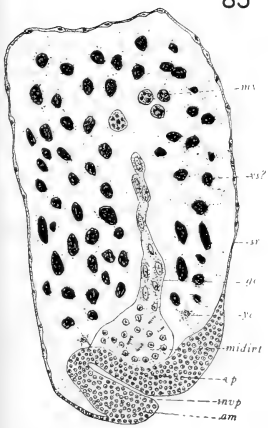
85 to 87 Oblique longitudinal sections of more older embryos.


83
sr
invp
yc


82
sr
bld
gc
midint


86
sr
ys?
yc
midint
mesg
ect


84
bld
ys?
gc


85
ms
ys?
sr
gc
yc
midint
ap
invp
am

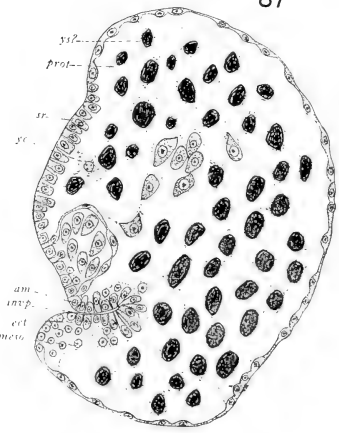
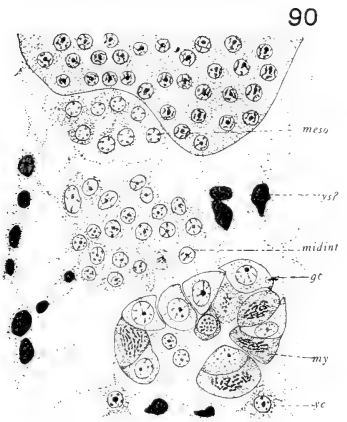
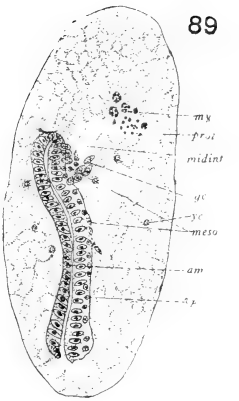

87
ys?
prot
sr
yc
am
invp
ect
mesg

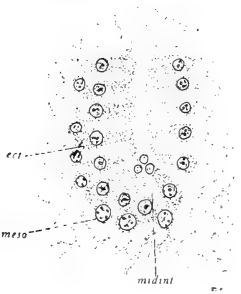
PLATE 13

EXPLANATION OF FIGURES

89 Longitudinal section of an egg of Pseudococcus.
90 A magnified view of figure 99 between *vp.* and *my.*
91 Posterior half of a longitudinal section of an early Lecaniodiaspis embryo.
92 A portion of figure 96, much magnified.


90
meso
ysf
midint
gc
my
yc


89
my
midint
gc
yc
meso
am


91
ect
meso
midint

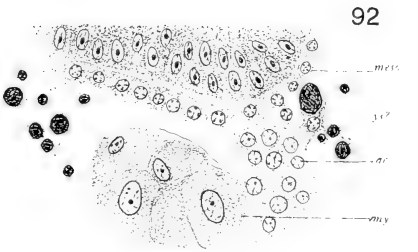

92
my

PLATE 14

EXPLANATION OF FIGURES

93 to 96 Four consecutive longitudinal sections of an embryo. Pseudococcus.

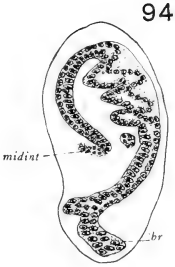
97 Transverse section of an embryo, Lecaniodiaspis, approximately in the stage represented in figure 89.

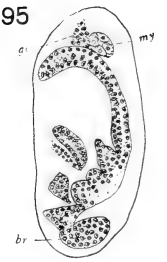
98 Oblique longitudinal section of an egg of Pseudococcus.

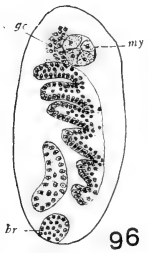
99 Longitudinal section of a Pseudococcus embryo.

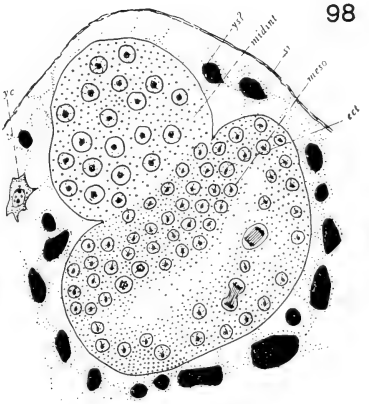
100 A portion of transverse section through the blastopore of Pseudococcus.

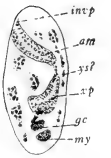

93
midint
br

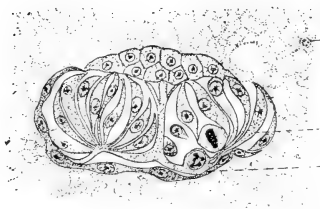

94
midint
br

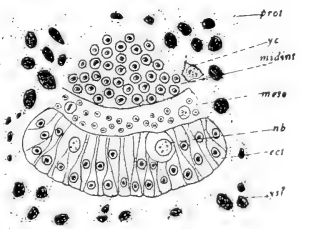

95
my
br


gc
my
br
96


98
midint
meso
ect


99
intp
am
ysl
ap
gc
my


97
yc
ect
am
prot


100
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yc
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meso
nb
ect
ysl

PLATE 15

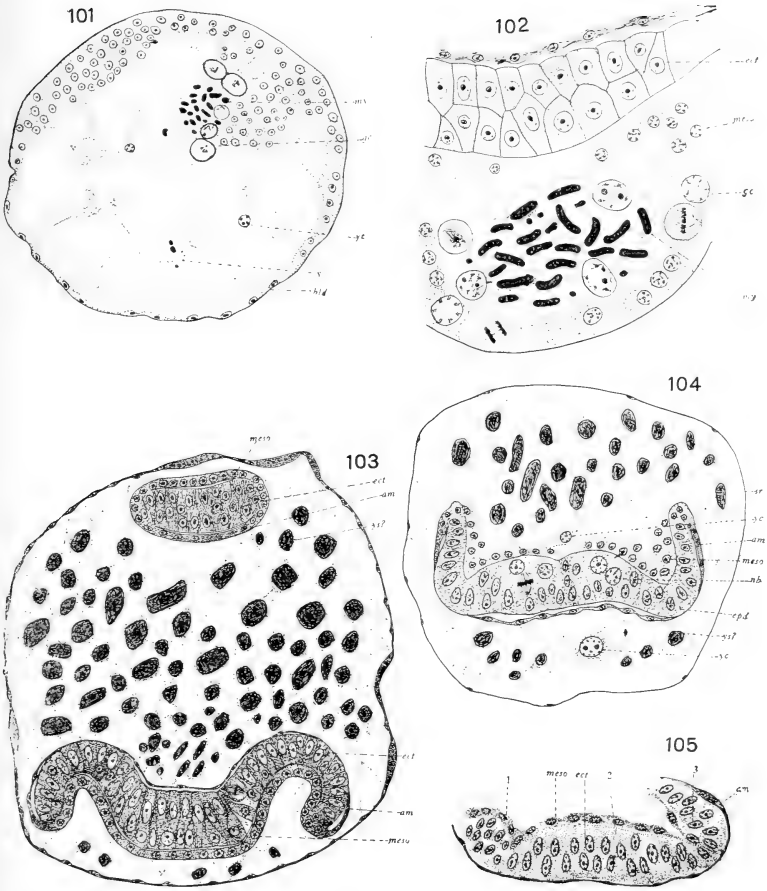
EXPLANATION OF FIGURES

101 Oblique transverse section through the posterior end of an egg of Icerya.

102 A portion of longitudinal section of an egg like the one represented in figure 66.

103 Transverse section through the second maxillae of an Pseudococcus embryo like the one represented in figure 50.

104 Transverse section through the second maxilla region of an embryo like that represented in figure 50.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mes
ect
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syc
meso
ect
1
2
3
am

PLATE 16

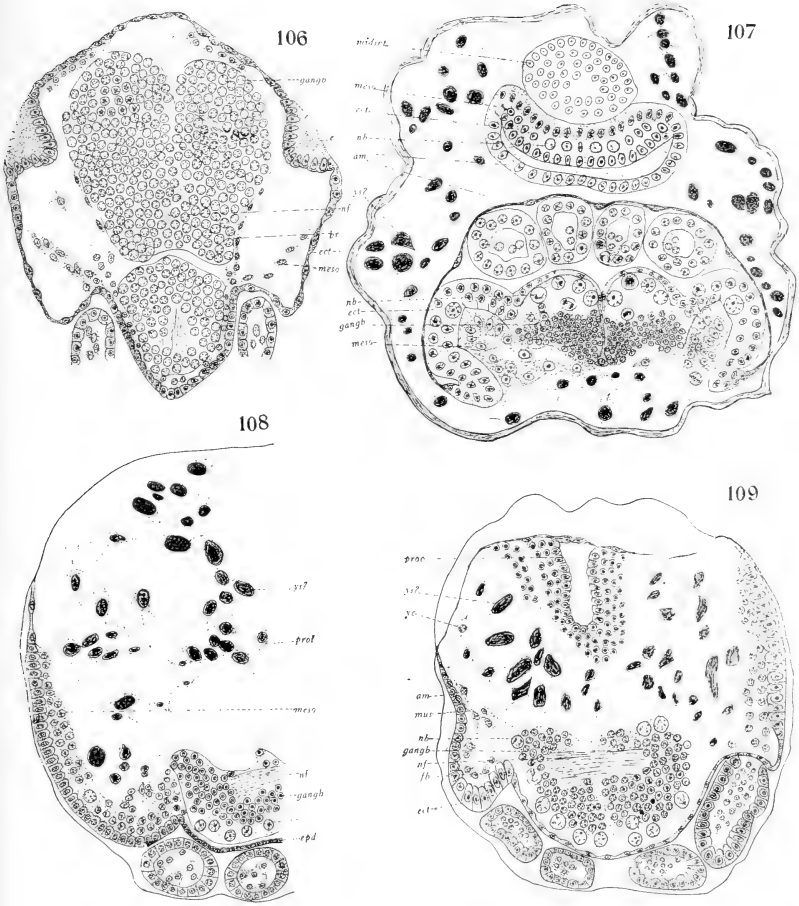
EXPLANATION OF FIGURES

106 Oblique frontal section of the embryo like the one represented in figure 55.

107 Transverse section of a Pseudococcus embryo somewhat older than the one represented in figure 55.

108 Transverse section through thoracic region of the embryo represented in figure 105.

109 Transverse section through the last thoracic region of an embryo somewhat older than the one represented in figure 55.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ys?
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ect
gangh
mesv
108
ys?
prol
mesv
mf
gangh
epd
109
proc
ys?
yc
am
mus
nb
gangh
mf
fb
ect

PLATE 17

EXPLANATION OF FIGURES

110 Longitudinal section through the brain of an embryo like the one represented in figure 51.

111 Transverse section through the thoracic region of a Pseudococcus embryo like the one represented in figure 72.

112 Transverse section through the salivary glands of Pseudococcus.

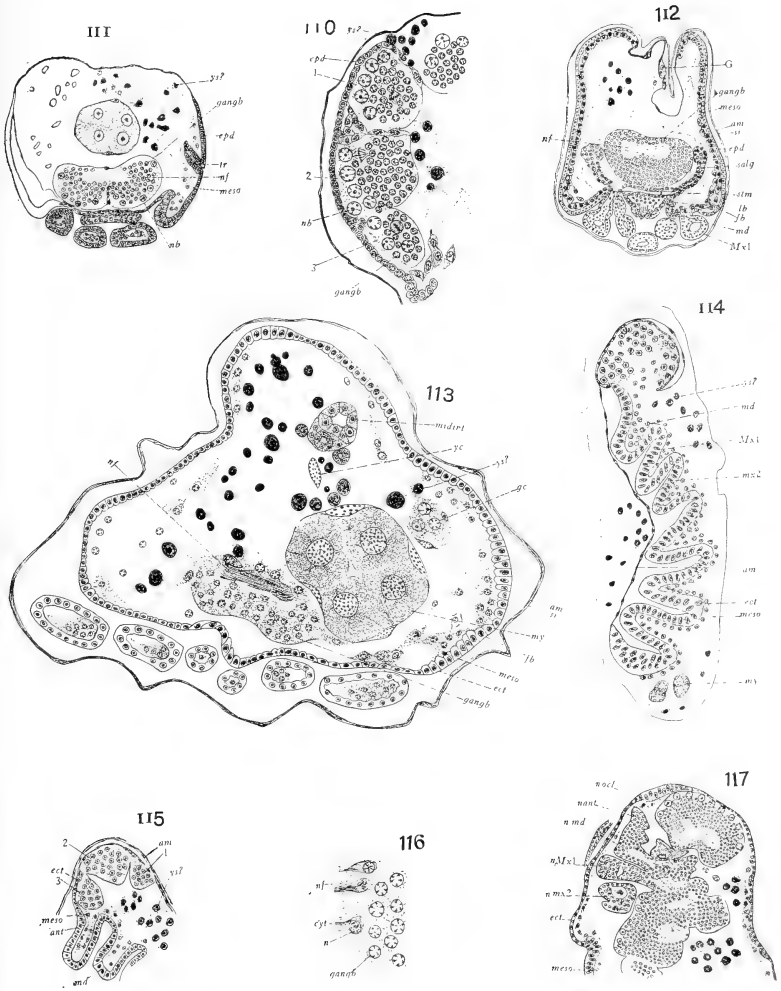
113 Transverse section through the third abdominal segment of the embryo almost ready to hatch.

114 Transverse section of a Pseudococcus embryo like the one represented in figure 51.

115 Another section of the same embryo as figure 114.

116 A portion of figure 117 magnified.

117 Oblique longitudinal section of an embryo.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2
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nf
epd
salg
stm
lb
fb
md
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midgt
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ys?
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gc
am
my
fb
meso
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meso
my
115
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am
ect
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ys?
meso
ant
end
116
nf
cyt
n
ganglb
117
mocl
nant
n md
n Mx1
n mx2
ect
meso

PLATE 18

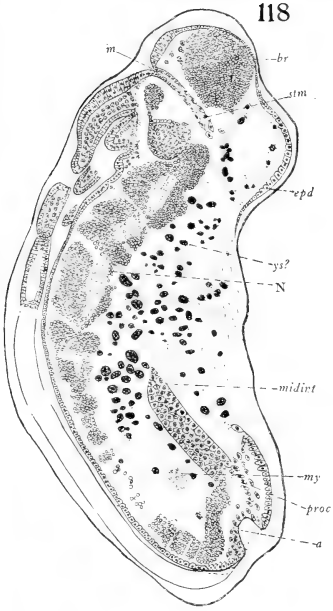
EXPLANATION OF FIGURES

118 Median longitudinal section of a Pseudococcus embryo a short time before the completion of the alimentary canal.

119 Longitudinal section of the embryo of Pseudococcus at the time of the completion of the alimentary canal.

120 Longitudinal section of an Iceryan embryo.

121 Transverse section of an Iceryan embryo like the one represented in figure 119.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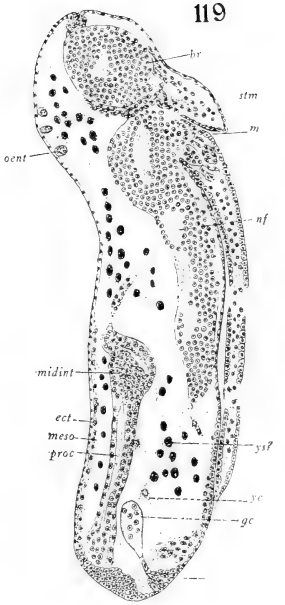
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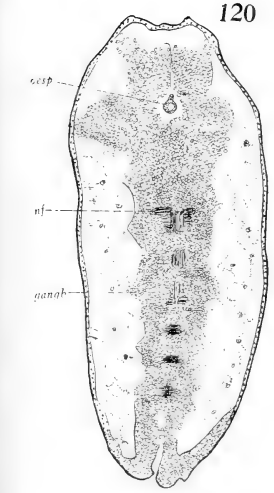
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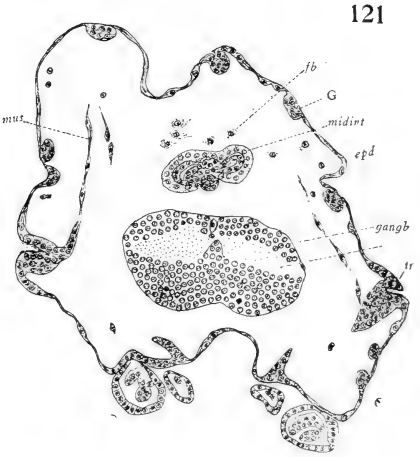

121
fb
G
midint
mus
epd
gangb
tr

PLATE 19

EXPLANATION OF FIGURES

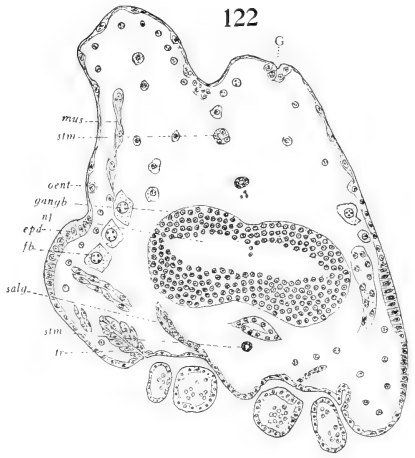
122 Transverse section of Iceryan embryo like the one represented in figure 119.

123 and 124 Hair glands of a newly hatched Pseudococcus larva.

125 Transverse section through the eyes of an embryo like the one represented in figure 119.

126 Caudal portion of an embryo Pseudococcus like the one represented in figure 70.

127 and 128 Stages of the growth of the rudiments of the midgut of Pseudococcus.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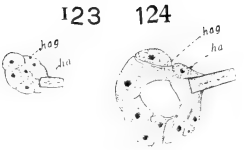
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124
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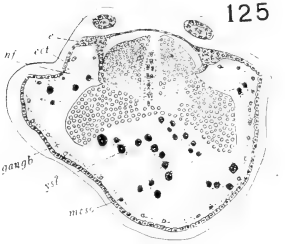
ha
hog
ha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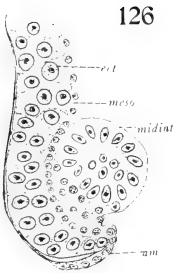
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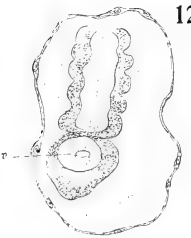
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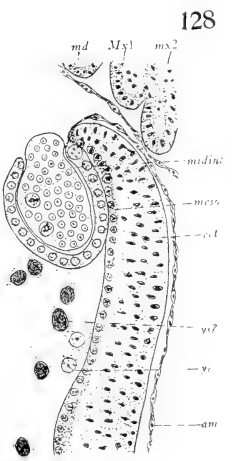

128
md
Mx1
mx2
midint
meso
ect
ysl
ve
am

PLATE 20

EXPLANATION OF FIGURES

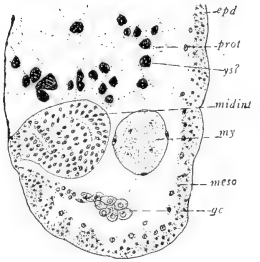
129 and 130 Stages of the growth of the rudiments of the midgut of Pseudococcus.

131 Longitudinal section of an Iceryan embryo a short time after the completion of revolutions.

132 Longitudinal section of an Iceryan embryo like the one represented in figure 119.

133 The colony of symbiotic parasites at the time of the hatching of larva.

129



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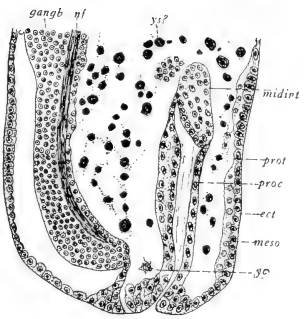

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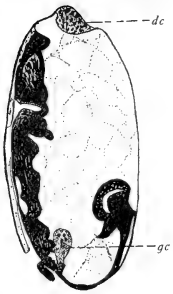

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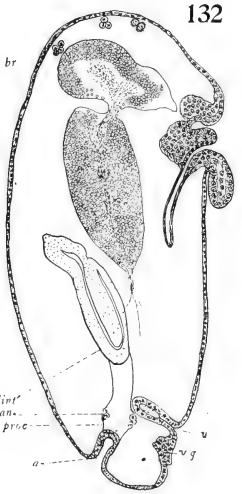

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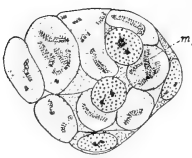

Resumen por el autor, Charles L. Parmenter,
Universidad de Pennsylvania.

Número de cromosomas y parejas de cromosomas en las mitosis somáticas de Amblystoma tigrinum.

El autor dá a conocer en el presente trabajo un estudio del número de cromosomas y sus relaciones de longitud en las células de varios tejidos somáticos de Amblystoma tigrinum, cuyos resultados suministran pruebas en favor de la teoría de la individualidad de los cromosomas En sesenta células pertenecientes a veinte y tres individuos diferentes el número de cromosomas es constantemente veinte y ocho. Las medidas lineales de los cromosomas de un número limitado de complejos seleccionados cuidadosamente indican que los cromosomas de una célula forman una serie duplicada en tamaño y forma, lo que presta apoyo a la suposición de que están formados por pares de cromosomas homólogos maternos y paternos. También existe una constancia aproximada en la relación de tamaño entre los pares de cromosomas de los complejos de diferentes individuos. Los datos mencionados favorecen la teoría de la individualidad de los cromosomas y no confirman el aserto de Della Valle, que supone que la variación del número de cromosomas es la regla y que las longitudes de dichos cromosomas en una célula se deben meramente a una casualidad.

Translation by José F. Nonidez
Carnegie Institution of Washington

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, OCTOBER 13

CHROMOSOME NUMBER AND PAIRS IN THE SOMATIC MITOSES OF AMBYSTOMA[1] TIGRINUM

CHARLES L. PARMENTER

Zoological Laboratory, University of Pennsylvania

THIRTY-SEVEN FIGURES (NINE PLATES)

CONTENTS

[1] Also known as Amblystoma.

INTRODUCTION

It is believed (McClung, '17, pp. 536–38) that the chromatin of an organism is, for the most part at least, the idioplasm, and consists of a definite linearly arranged series of differentiated materials which is perpetuated from generation to generation. The chromosomes which are essentially constant in number in an individual are thought to constitute the visible mechanism for this perpetuation. This conception is known as the theory of the individuality of the chromosomes, which is quite generally accepted by all who have an intimate acquaintance with chromosome behavior. However, there are a few not so acquainted who strenuously oppose the theory.

Among these is Della Valle ('09, '11, '12), who presents some data and a large amount of discussion in an effort to disprove this theory upon the claim that the chromosome number in an individual is not constant, but is simply the quotient of the quantity of chromatin divided by the average size of the chromosomes. This removes from them any constancy of organization and contradicts the above theory. These observations have been cited by other opponents of the theory as cytological evidence in favor of their contentions. Della Valle's conclusions are based upon observations made upon dividing cells of the peritoneum and blood-cells of Salamandra maculosa, together with a large amount of data taken from the observations of others.

Meves ('11) and Della Valle ('12) further oppose the theory upon the basis of linear measurements made upon the spermatogonial and somatic chromosomes of Salamandra maculosa in denying Montgomery's ('01) and Sutton's ('02) claim that the chromosomes occur in pairs whose homologues are of equal length, and that approximately constant size relations among chromosomes are maintained from one cell generation to another.

In the spring of 1916 I was fortunate in obtaining peritoneal and other somatic tissues of Ambystoma tigrinum. This made it possible to repeat Della Valle's observations upon the somatic

cells of the same and other tissues of this closely related species and thus to determine whether such a variation as he claims is present in the somatic tissues of other Amphibians. Also the chromosomes of some cells in my material are sufficiently favorable for measurements to permit a reconsideration of their length relationhips.

Since this paper is regrettably controversial, it is necessary to give careful attention to all the methods and conditions under which the preparations and the observations were made. Della Valle also lays much emphasis upon this point, and therefore considerable space is devoted to this problem.

For facilities in collecting and preparing this material I am indebted to the courtesy of the Department of Zoology of the University of Minnesota, and to Prof. C. P. Sigerfoos I owe the loan of several very excellent preparations. The work was done under the direction of Prof. C. E. McClung, of the University of Pennsylvania, toward whom I feel especially grateful for constant encouragement and valuable criticism, and for his characteristically generous and kindly interest at all times. I am also greatly indebted to other members of the department, especially to Dr. Eleanor Carothers and Dr. D. H. Wenrich, for helpful suggestions and very painstaking criticisms.

TECHNIQUE

The material used was obtained during the spring of 1916 from larvae of Ambystoma tigrinum, which were abundant in the ponds and lagoons near the College of Agriculture of the University of Minnesota. Mitotic figures in epithelial cells of the tail, gill plates, and lung, and of the endothelium from peritoneum and mesentery were studied.

Tail epithelium

Very excellent preparations of this tissue were kindly loaned me by Prof. Charles P. Sigerfoos, of the University of Minnesota. These were made from the tails of larvae $\frac{3}{4}$ to $1\frac{1}{2}$ inches in length obtained during the last of May and the first of June during several years.

The living larvae were thrown into Flemming's stronger solution. After about four hours of fixation, the tails were split dorsoventrally[2] into two thin plates of cells. These two plates of cells were then fixed twenty hours longer. After washing in running tap-water for twelve hours or more, the pieces were stained in toto in Heidenhain's haematoxylin, carefully dehydrated, and cleared in xylol and mounted in damar.

Gill-plate epithelium

The most successful gill-plate preparations were also obtained from larvae $\frac{3}{4}$ to $1\frac{1}{2}$ inches long. In each larva there are eight gill plates, one subtended from each gill arch and another behind each posterior gill cleft. These gill plates contain very numerous mitotic figures. They are composed of two epithelial lamellae with connective-tissue cells and capillaries lying between them. The two layers, unseparated, are so thin that they give very excellent preparations.

The material was fixed in situ by dropping the living larvae into the Flemming's stronger solution as soon as they were taken from the net. They were fixed in situ twenty-four hours and

[2] Haecker ('99) describes a very successful method of separating these two plates of cells. The posterior end of the larva is cut off after fixation just in front of the cloaca. With a sharp scalpel the thick cephalic end of the tail is split dorsoventrally through the middle of the vertebra to a depth of an eighth of an inch or more. By grasping with the forceps the ends thus made free, the two layers of epithelium can be pulled apart in a manner similar to separating two sheets of fly-paper with adhesive surfaces sticking together. Professor Sigerfoos advises separating the two layers after about four hours of fixation and then allowing to fix about twenty hours longer.

The numerous large mitotic figures in various stages with clear cell walls which can be studied without an immersion lens makes this material excellent for the class-room. The gill-plate preparations are equal or superior to those of the tail epithelium. Those of larger larvae are too thick when mounted in toto, but give very satisfactory preparations when separated. Peritoneal preparations of larger larvae contain fewer mitoses and the cell walls are indistinct. However, preparations can be made from the more rapidly growing shorter larvae from which the gill-plates were taken and would probably contain more divisions. Preparations of Ambystoma punctatum are less favorable than those of A. tigrinum because there are fewer figures, more pigment cells in the tail epithelium and the gill-plates are small and thicker.

washed in running tap-water. The larvae from which the gill-plates were taken were fixed for other purposes, and no special effort was made to insure good fixation of the plates. They were preserved in position in 5 per cent formalin, which was later gradually replaced with 80 per cent alcohol.

The fixed gill-plates were carefully removed from the larvae in 80 per cent alcohol and were left attached to the gill arches, which, in subsequent handling, were grasped by forceps to prevent injury of the plates. Hydrogen peroxide was added to the 80 per cent alcohol drop by drop through a fine glass capillary siphon until the solution amounted to equal parts of each. In this the plates were bleached for four to twelve hours and then transferred to the mordant by the above-mentioned drop-process and stained in iron haematoxylin. They were dehydrated by this drop method, cleared in cedar-wood oil followed by xylol, cut from gill arches, after being transferred to the slide, and then covered with damar and a thin cover-glass. While the damar was hardening they were kept for twenty-four hours or more under slight pressure to insure flat preparations.

The peritoneum, mesentery, and lungs

These preparations were made from larvae 3 to 4 inches long. All the tissues of a given individual were not only fixed together in the same fixative for the same length of time, but also received the same treatment in all subsequent processes. They were put into the fixatives within an estimated maximum of two minutes after the first incision. Two methods of procedure were used in preparing these tissues for fixation:

1. In order to avoid any possible unfavorable effect of captivity, the tissues were fixed in situ in the field as soon as the larvae were taken from the net. The animals were prepared for fixation as follows: With sharp scissors the body wall was cut open along the midventral line and also lateral incisions were made on each side at right angles to the first incision behind the pectoral girdle and in front of the pelvic girdle, so that the two halves of the body wall fell away from the viscera and opened

wide the body cavity. The folds of the viscera were pulled apart and the whole larva was plunged into the fixative. This secured immediate and uniform fixation. The operation requires less than a minute and the incisions are apparently painless, for the larva does not often struggle.

2. The body walls, lungs, and viscera were removed from the body of the larvae before fixing, either in the field or at the laboratory. The peritoneum was fixed in situ on the body walls. Only normally inflated lungs were used, and these were ligated anteriorly before removal from the body to prevent them from collapsing. After fixing one or two hours, they were cut into two or more flat longitudinal strips and returned to the fixative. The mesentery, attached to the intestine, was spread out flat on a piece of glass and the whole immersed in the fixative with the tissue beneath.

Fixatives

The fixatives used were: 1) Flemming's stronger solution, thirty hours; 2) Bouin's solution, forty-three hours; 3) Bouin's solution, to which was added $1\frac{1}{2}$ grams of chromic acid crystals per 100 cc., twenty to twenty-four hours; 4) Hermann's solution with two parts of osmic acid (Lee, '13, p. 38) twelve to eighteen hours; 5) a solution of saturated picric acid 75 cc., formalin 15 cc., glacial acetic acid 10 cc., urea crystals 2 grams, thirty to forty-three hours. The urea should be added gradually to the solution warmed to about 40°C., otherwise a precipitate is formed.

It is a difficult matter to decide which solution gave the best fixation. The prettiest cells were fixed in Hermann's and the chromic acid modification of Bouin's fluid. However, the peritoneum preparations of the osmic fixatives were a little thicker and less transparent than the others. If any fixative should be exclusively chosen, I believe it should be the chromic acid modification of Bouin's solution, because of its excellent fixation, convenience, and economy.

The peritoneum was removed as follows: The two sides of the body wall were detached by an incision along the back

close to the spine. Under the binocular lens, in water, the peritoneum was carefully loosened from the underlying tissue by scraping it with a sharp scalpel, first along the edge cut from the back. Sections of this loosened edge were then grasped by the forceps and relatively large sheets were easily pulled off from the underlying tissue. The peritoneum covering the dorsal and lateral portion of the body wall is deeply pigmented and to it adhere considerable muscle and connective tissue when the peritoneum is removed. This portion was grasped with the forceps in removing the peritoneum from the body wall, as well as in all subsequent handling. Consequently the cells in the ventral transparent region available for study have been undisturbed by instruments. However, there still remains a possibility that the strain of pulling the peritoneum loose might disturb some cells.

Peritoneum fixed in Flemming's and Hermann's solutions was stripped from the body wall after four hours of fixation and then fixed twenty hours longer. That treated with the various picric acid mixtures was stripped immediately after fixation. However, the peritoneum fixed in the chromic acid modification of Bouin's solution may be preserved in alcohol for as much as a year before stripping. That of Ambystoma punctatum, fixed in Flemming's stronger solution and preserved in 5 per cent formalin, can be stripped at least six months after fixation.

Material fixed in osmic acid fluids was washed five to fourteen hours in frequent changes of tap-water. Picric acid preparations were gradually transferred to 70 per cent alcohol, beginning with 10 per cent and progressing through successively stronger grades differing by 10 per cent. They remained in each grade five to ten minutes. The tissues remained in 70 per cent alcohol containing a few drops of saturated aqueous lithium carbonate solution until the picric stain was removed, and before staining they were returned to water by reversing the above process. All of the material was stained in Heidenhain's haematoxylin after mordanting in a $2\frac{1}{2}$ per cent solution of iron alum for four to six hours. No counterstains were used.

Dehydration was accomplished by passing the material through the above grades. The fluids were removed from, and added to, the containers without handling the material. Alcohols were followed by half xylol and half absolute alcohol, and finally by xylol.

The pieces of peritoneum were transferred from xylol to a slide where the above-mentioned pigmented area, with the attached muscle fibers, was removed quickly with a sharp scalpel just before mounting. After mounting in damar under a coverglass, they were put under a light pressure for twenty-four hours or more while drying to insure as flat a preparation as possible.

OBSERVATIONS

It should be emphasized that the preparations upon which these observations were made are unsectioned surface membranes. This makes it possible to study the mitotic figures with the confidence that all of the chromosomes are present and that none have been cut and are being counted more than once. This is an important consideration in determining whether the number of chromosomes is constant.

A. The number of chromosomes

There are twenty-eight chromosomes in the somatic complexes of Ambystoma tigrinum. In forty-five unquestionable enumerations and in eighteen which contained either one or two chromosomes that might possibly be considered subject to interpretation, there are none which vary from twenty-eight. In three complexes, because of the alternative interpretations possible at one or more points, the number cannot be definitely determined and is interpreted to be either twenty-seven or twenty-eight. The fact that these numbers are so close to twenty-eight is strong evidence that these cells contain the usual number of chromosomes.

The counts as indicated in the accompanying table have been obtained from twenty-three different individuals varying in age

approximately from six to ten weeks. The preparations of tail epithelium loaned by Professor Sigerfoos were taken from a collection which he has been accumulating for a number of years. It is probable, therefore, that the counts in these preparations represent the chromosome number present during a series of years and that the number is constant from year to year.

Table showing for each tissue studied, the number of different individuals represented and the number of complexes with their distribution into classes as described on page 178. The total number of different individuals represented is twenty-three

TISSUE	NUMBER OF INDIVIDUALS	CLASSES			TOTAL
		I	II	III	
Peritoneum	7	14	6	1	21
Mesentery	1	1	0	0	1
Lung	4	7	2	1	10
Tail epithelium	5	9	4	0	13
Gill plates	8	14	6	1	21
Totals		45	18	3	66

a. Method of determining number. Since one of the chief purposes of this study is to determine accurately whether there is any variation in the number of chromosomes, considerable care has been taken to eliminate from the evidence every possible source of error. An important part of the presentation of this evidence is, then, a concise description of the exact procedure employed in obtaining it.

1. Procedure. In order to avoid overlooking any mitotic figures, the entire surface of every piece of tissue was completely surveyed systematically before beginning to count any of the chromosomes in any of the complexes. The survey was accomplished with a 4-mm. objective and an 8× ocular supplemented by a mechanical stage.

In determining the number of chromosomes in each complex, a camera lucida sketch of it was first made at a magnification of 2633 diameters. This sketch was carefully compared with the cell in order to make certain that no errors had been made in

sketching it. The chromosomes were then numbered consecutively, the number being placed on both ends of each chromosome. This method avoided any possibility of overlooking any chromosome or of counting any chromosome twice.

2. Clearness and classification of the complexes. All the complexes counted were polar views of late prophases and of metaphases and have been divided into three classes on the basis of their clearness. The first class consists of forty-five complexes in which every chromosome was so clearly separated from adjacent chromosomes that it could be optically traced continuously over its entire length, without losing sight of it at any point. Only the counts from complexes of this group are submitted as data which are unquestionably free from objection and uncertainty.

In the second class of cells there are eighteen complexes in which the chromosomes are all exactly as clear as those of the first class, with the exception that either one or two chromosomes cannot be clearly traced over their entire length as they could be in class I and therefore might possibly be hypercritically considered to necessitate interpretation.

The three cells of the third class differ from those of the second class in that they each contain places in which the number of chromosomes cannot be determined with confidence and consequently are actually subjects for interpretation.

Complexes of the first class. Complexes of this class are represented by figures 1 to 8 which have been made in carbon and are attempts to represent the actual appearance of the chromosomes and their relative positions in the complexes. Representative cells from each of the tissues studied, except the mesentery and lung, have been so drawn. Other complexes of this group have been outlined in ink, figures 9 to 20, to give a further assurance of the nature of the complexes constituting this class of conditions.

Since it is impossible to represent chromosomes in a drawing as clearly as they are seen in a cell, it is necessary to consider briefly this situation in order to prevent misunderstanding, and incorrect impressions concerning the clearness of the cells and

the faithfulness of the description of the conditions under which the number of chromosomes was determined. The difficulty lies in the necessity of representing on a plane surface chromosomes which in the cell occupy several levels. The effect can be produced by shading, but at the same time at points where chromosomes cross or overlap each other for various distances they might create the impression in the drawing that they cannot be "optically traced continuously over their entire length." There are such cases in every drawing. This is especially true of the late metaphases of the tail epithelial complexes (e.g., figs. 7, 8) where every chromosome in the cell can be clearly and faithfully traced as described above.

There is also the condition in which parts of the same chromosome are so related to one another that their appearance in the drawings might create a doubt as to their clearness in the cell. Examples of this are represented in figures 6 and 8, chromosome 'a,' in which the two arms of the same chromosome turn abruptly upon one another and the appearance might be subject to the criticism that there are two different chromosomes involved—a portion of one lying exactly upon another with their ends terminating at the same point. Such cases were carefully examined end the two arms can clearly be seen to follow into each other.

In four of this first class of cells there is another condition that needs mention. These cells contain one or two chromosomes which appear to be broken into two parts (e.g., figs. 19 and 20, *f*). The parts in each case are separated by very short spaces and are exactly in line with each other. Della Valle ('09, fig. 11) shows two cases of this sort as one chromosome, but discusses them (p. 116) as uncertain. That there is a single chromosome concerned in each of these cases is further evidenced by the fact that there are twenty-one similar cases in other cells of this class (e.g., figs. 5, 7, 14 and 15, *f*) and thirty-five cases in cells of class II in which the parts are connected by various amounts of chromatin. In some instances the connection is seen as faintly staining chromatin, in others as a single or double darkly stained thread.

Complexes of the second class. In fourteen cells of this class there is one point in one chromosome and in four cells there is one point in each of two chromosomes which, to persons hypercritically inclined, might possibly appear uncertain. To one acquainted with the material, each of these points is entirely clear, and even when accepted as subject to interpretation it is very plain how the interpretation should be made—so plain that I am certain that the count of twenty-eight chromosomes is accurate and dependable. But for the sake of unquestionable fairness I have placed these cells in a separate group. As to the exact nature of the interpretations in these eighteen complexes, four of them have some small portion of only one chromosome so covered by others that it cannot be traced over its entire length without losing sight of it as stated above (p. 178). Two other cells had two chromosomes of this nature. Five complexes have a single chromosome lying in such a relation to another chromosome that it might possibly be interpreted as a part of the other chromosome (e.g., fig. 23, chromosome *i*), and in three more cells there were two such chromosomes. In the remaining five complexes a single chromosome was so situated or otherwise involved, that it might be interpreted that there were two chromosomes present (e.g., fig. 21, *i*).

In considering all the interpretation possible in each of these eighteen cells the minimum number in any one of them would be twenty-seven and the maximum number thirty. Even granting this much variation, it is far removed from that expected in a series of chance variants as Della Valle claims them to be.

The points in question were sketched as described above before the chromosomes were counted, so that the determination of the number of chromosomes was not influenced, either consciously or unconsciously, by a knowledge of how many chromosomes were present or by how they should be sketched in order to produce the expected number. This procedure and the fact that the number counted always agreed with the number present in the forty-five cells of class I make it practically certain that the enumeration is correct. It should be emphasized again that these cases are only subject to question when hypercritically

considered and would otherwise constitute a part of class I. In fact, an experienced cytologist of this laboratory, in examining these, without a knowledge of the number of chromosomes present, could see no reason for considering them as subjects for interpretation, and it seems almost absurd to place them in a separate class.

Complexes of the third class. There was a very large number of cells which were beautifully clear everywhere except in regard to one or two chromosomes. However, only three of these were sketched, because the number of clear counts was so large that an increased number of these uncertain counts is of little value.

Each of the three cells drawn contains two points of uncertainty as to whether there are one or two chromosomes present. The number is interpreted as either twenty-seven or twenty-eight. The minimum number of chromosomes possible of interpretation in one cell is twenty-six, the maximum is twenty-eight; in the other two cells the minimum is twenty-seven, the maximum is twenty-nine.

These three cases were interpreted while the sketch was being made and before it was known how many chromosomes were present. It is not true, therefore, that the interpretations were prejudiced nor that any cases which did not agree with the expected numbers were cast aside and consequently ignored. On the contrary, they are here included as part of the evidence in forming the conclusions drawn from this study.

Rationally considered, then, of the cells sketched there are sixty-three in which the enumeration of chromosomes is accurate and dependable and three in which there are unavoidable interpretations necessary. These sixty-six complexes constitute very strong evidence that the number of chromosomes in Ambystoma tigrinum is constant.

b. Possible variation in number in uncounted complexes. As to whether or not there was any variation in chromosome number in this species can be judged from the results obtained from the sixty-six cells which were studied. If as few as 2 per cent of the total complexes studied varied from the usual number, at least one of these should have made its appearance. Furthermore,

Della Valle ('09, p. 117) claims that variation of chromosome number is probably a general law and (p. 120) that his counts strikingly bear out the expectation expressed by Newton's theoretical binomial curve. Were this the condition in Ambystoma tigrinum, a good proportion of the sixty-six complexes should have shown variation in number. Since no variation was found, it is safe to conclude that there is none in the cells that could not be counted.

c. Abnormal complexes. Seven apparent variations from the usual number were found. These were groups of chromosomes in which the number was clearly other than twenty-eight (figs. 22, 24, 25, A.B., 26, A.B.). But when these groups are thoroughly analyzed it is certain that they are nothing else than cases of a very unusual behavior of four cells and do not constitute a variation from the usual number of chromosomes.

Figure 22 shows a peritoneal cell which has lost a part of the chromosomes. Chromosome *a* is but part of a chromosome, showing very unmistakable evidence that a portion of it has been broken off and there is a conspicuous depression in the tissue from which it is evident that the remainder of the chromosomes of this cell have been lost. The cell lies close to a tear in the peritoneum. It is a bare possibility that the tear and the loss of the chromosomes is due to the same cause.

The second case is a very early metaphase from the peritoneum. It consists, as represented in figure 24, of one group of twelve chromosomes and another group of sixteen immediately adjacent to it. These two groups and figures 2 and 20 are very similar to Della Valle's dicentric cell ('09, fig. 6) and Flemming's ('91) figures 31 to 39, table 40. A study of these two groups makes it practically certain that they are separated parts of one and the same cell. This is evidenced by the following facts: 1) these two groups together constitute the normal number twenty-eight. 2) The chromosomes of both groups of cells are in the same stage of mitosis. 3) Both groups represent a half circle and indicate strongly that they are separated parts of one cell which have rotated a total of 180° to their present positions. 4) When these chromosomes are arranged side by side linearly they

form a series (fig. 31) like that (figs. 27 to 30) made by a similar arrangement of the chromosomes of normal cells (figures 1 and 3). The length relationships of these chromosomes as shown graphically in figure 37 are practically identical with those of normal cells (figs. 33 to 36). Unfortunately, the cell walls are not visible. 5) Both of the homologues of chromosome pairs, as determined by measurements and indicated in figure 24 by a duplicate series of numbers, in some cases are found in the same separated part of the cell and in other cases one homologue is found in part *a* and the other in part *b*.

The third case is a compact metaphase in the epithelium of the lung (figs. 26 A and B) and is similar to the second case. These two groups are somewhat more separated than those of figure 24. Figure 26 B represents the chromosome number and characteristics and figure 26 A shows the relative positions of the two groups omitting some of the chromosomes in *a*.

That these two groups of chromosomes are parts of the same cell which have become separated is made highly probable by the following facts: 1) As in the second case (fig. 24), the two groups are near together, one containing eight and the other twenty chromosomes—a total of twenty-eight. 2) The chromosomes are in the same stage of mitosis, the chromatids of those of *b*, however, being separated a little more than those of the twenty chromosomes in *a* which may be due to a less crowded condition. 3) The two groups are practically of the same diameter and of the same shape. An outline of *a* on transparent paper can be perfectly fitted to *b*. 4) These chromosomes also form a linear series of lengths (fig. 32) similar to those of normal cells. 5) Group *a* is not a complete cell because the cytoplasm can be seen only below the chromosomes, while above the chromosomes are bare. The boundaries of the cytoplasm of *b* cannot be seen. 6) The homologues of the chromosome pairs are numbered and distributed in the two groups like those of figure 24.

The fourth case is the peritoneum of another individual. It evidently is a cell which has been divided into two parts like those of cases 2 and 3. Figure 25 A is a camera-lucida drawing

representing the relative positions of the two groups and figure 25 B shows the chromosomes enlarged and numbered consecutively. As in case 3, the two groups are not immediately adjacent, but are separated by the longer diameter of a resting nucleus.

In group *a* there are eleven chromosomes. In the other group there are apparently seventeen, but unfortunately in this second group the chromosomes are so overlapped at one point that they cannot be counted with confidence. There are, however, thirteen chromosomes which can be clearly delineated and the interpretation that there are four chromosomes in the group (14 to 17) which so badly overlap is likely correct. The total number of chromosomes in the two groups is then probably twenty-eight.

The chromosomes are so much foreshortened, and at the above-mentioned point so crowded, that I have not attempted to measure and arrange them in a series as was done for the chromosomes of figures 24 and 26. However, a glance at figure 25 B shows that such a series might be arranged.

The shape and size of both groups of chromosomes, and of the cytoplasm about them, are such that one can be fitted upon the other. Although these two relations are not positive evidence they indicate that one of these groups, possibly the smaller, has been separated from the other.

To summarize, it may be said that in the first case considered (fig. 22) it is certain that the smaller number of chromosomes is due to a loss of a part of the chromosome complex from the cell. Although the facts stated for cases 2, 3, and 4 may not be considered absolute proof, they do constitute a very strong probability, which closely approximates a proof, that in each of these cases a cell has been separated into two parts.

B. Somatic chromosome pairs

a. Introductory statement. Since Van Beneden's ('83) hypothesis that one-half of the chromosomes of an individual are of paternal origin and that one-half are of maternal origin there

have been many confirmatory observations and some that oppose it.

Montgomery ('01, p. 220) advanced evidence that for each of the chromosomes of maternal origin there is a homologous mate among the chromosomes of paternal origin, and that these homologues unite during synapsis. He also maintained that these pairs[3] can be recognized in the spermatogonia. This view has been supported by many authors. Among these are Sutton ('02), who compared numerous camera-lucida drawings of spermatogonial complexes of Brachystola magna; Meek ('12 a), who measured the lengths of spermatogonial chromosomes of a somewhat wide range of animals, and Hance ('17 b, '18 a), who made linear measurements on the germinal and somatic chromosomes of the primrose, Oenothera scintillans and the pig. On the other hand, Meves ('11), on the basis of measurements made upon the spermatogonial and somatic complexes of Salamandra maculosa, fails to confirm the claim for the former and denies it (p. 282) for the latter. Della Valle ('12), who measured the chromosomes of peritoneal cells of the same form, also denies the existence of pairs.

Some of the somatic cells studied in Ambystoma tigrinum are quite favorable for a linear measurement of chromosomes, and these complexes have been used to obtain further data upon the query as to whether the chromosomes of the somatic cells form a duplicate series (based upon length and form) as is shown by their progenitors in the germinal line during the maturation period.

b. Mensuration. Since the possibilities of error in measurements are so great, it is necessary to consider the conditions

[3] The two mates constituting a pair are usually of equal length, so that homologues may be recognized by such equality. In some cases, for example, in the Diptera, Stevens ('08, '11), Metz ('14, '16 a and b), Holt ('17), Whiting ('17), Hance ('17), the two members lie near each other or even closely approximated in the spermatogonia and somatic cells, while in many other cases, for example, in Orthoptera and Amphibia, the homologues may be widely separated in these cells. In the present paper the term 'pairs' will refer to the two chromosomes which are homologues as determined by length and form regardless of the relative position in the cell.

under which these measurements were made in order to judge their value correctly.

1. Type of cells. Only cells were used in which every chromosome was perfectly clear and, except as noted (p. 189), lay exactly level in the equatorial plane throughout their entire length. Only three cells (figs. 1, 3, and 9) of this quality were available, and these were polar views of early metaphase stages in cells of the peritoneum and lung. The chromosomes of one other cell (fig. 10) approximated this condition and were also measured. The care with which these cells have been chosen may be judged from the fact that they were the only suitable cells in material from over one hundred larvae containing large numbers of division figures. In material with chromosomes so long and so numerous it is not surprising that so few cells were perfect enough for measurement.

2. Method. In addition to choosing cells with chromosomes of the above character, three different camera-lucida sketches of each chromosome were made on different days with extreme care at a magnification of 2633 diameters. Each of these sketches was measured three or more times along the median line with an Ott compensating planimeter modified for this purpose, or with an opisometer. These nine determinations obtained for each chromosome were averaged to represent its length. This method is important because the extremes of these nine measurements in about one-fifth of the cases may differ 1 mm. from the average (and occasionally more). This demonstrates that one measurement upon a single drawing might give rise to an erroneous difference in the lengths of the homologues of some pairs ranging from 1 to 2 mm., the actual amount depending upon the respective errors in each homologue. Averages largely eliminate this error.

3. Sources of error. The various sources of error may be classified in three groups: 1) instrumental errors, 2) personal errors, 3) errors inherent in the condition of the material.

In the first place, it should be emphasized that no attempt has been made to determine the actual length of any chromosome. These measurements have all been made on the drawings

described above and have to do only with relative lengths. This fact eliminates at once a number of errors which would otherwise be very serious.

The instrumental errors. The possible instrumental errors are *a*) failure to maintain a critical illumination, *b*) failure to maintain a constant wave length of illumination, and *c*) errors inherent in the planimeter and opisometer.

It so happened that the strongest light was obtained a little above the point of critical illumination, and might therefore cause an error in measurement. However, several chromosomes were drawn a number of times under both conditions and no perceptible difference was observed. Any slight error overlooked would be equal in the homologous chromosomes and would not interfere with a relative measurement.

Farmer and Digby ('14) state that errors can arise from the use of varying wave lengths of light. The same optical equipment and illumination were maintained in all of my operations so that relative values were unaffected.

In making measurements with the planimeter the polar arm was held rigidly stationary in two grooved blocks so that the tracing point moved around in a circle having a diameter of 33 cm. The sharp tracing point was kept upon the median line of the chromosome figure by moving the drawing around (without slipping) into line with the path of this tracing point, which was used as a pivot for orienting the drawing. A constant, representing the value of each of the divisions of the vernier, was determined by measuring a series of known distances on a straight line.

The accuracy with which this instrument was operated is indicated by the fact that the average difference between the extremes of measurements made upon each of several drawings is 0.3 mm., the standard deviation, computed from the combined measurements of several drawings, is 0.17 mm. The measurements, obtained more quickly with the opisometer, are slightly less accurate, the average of the above extremes and the standard deviations being 0.4 mm. and 0.26 mm., respectively. Finally, the average of the nine measurements made upon the three drawings of each chromosome reduces this instrumental error

to approximately zero. This error is, of course, inherently included as a part of the personal error discussed below.

Personal errors. Probably the greatest personal error was due to inaccuracies in making camera-lucida drawings. To reduce this error to a minimum, each chromosome, as stated above (p. 186), was drawn three times with extreme care. These sketches were made at the same point on the drawing-board so that any error due to different drawing distances and consequent differences in magnification was eliminated. The estimated median line of the sketch, upon which the measurement was made, was indicated with a lead pencil. The average deviation from the mean of the nine measurements is 0.6 mm. and the standard deviation, computed from combined measurements of several drawings, is 0.37 mm., which indicates that the instrumental and personal errors in the average of these measurements are practically zero for relative purposes.

Errors due to conditions inherent in the material. This class of errors is much more important than the preceding. The errors of this kind are an unequal shortening of the whole chromosome and a foreshortening of parts or all of the chromosome. Measurements made without very careful attention to foreshortening are of questionable value, for small amounts can give rise to large errors, especially in short chromosomes. Shortening is caused by a twisting of the chromatids about one another (figs. 1 to 8, 27 to 30). The amount of shortening in each twist of the chromatids, as determined by computation,[4] at the mag-

[4] The amount of shortening in each twist of a chromosome was determined by adding together the separately computed amounts of shortening due to the lateral deviation of the chromatids and that due to the vertical deviation of the chromatids. The shortening in each twist due to the lateral deviation was computed by averaging the lengths of the two chromatids of a chromosome and substracting the length measured upon the median line of the whole chromosome. This total difference divided by the number of twists is 0.2 mm., which is approximately the amount of shortening due to the lateral deviation in each twist. In determining the amount of shortening due to the vertical deviation of the chromatids, the width or thickness of the chromatid, as determined with an ocular micrometer was assumed to be the amount of vertical sag of the chromatid in each twist. This thickness multiplied by the magnification amounted to 1 mm. This was used as the altitude of a right triangle, the base of which represented half of the measured longitudinal length of the shortened part, and the hypotenuse of which then represents very closely one-half of the true length

nification of the drawings (2633 diameters), amounts very closely to 0.4 mm. However, the effect of this condition is either completely or largely neutralized by equal or nearly equal amounts of twisting in the homologues of each pair. The maximum amount of error due to this cause may be judged by an examination of figure 28 which contains the most twisting. In this cell there are seven pairs in which the amount of twisting is equal and the error completely neutralized, two pairs containing an error of 0.4 mm., two pairs with 0.8 mm. error, one with 1.2 mm., and two pairs in which it is uncertain. Since these errors that occur at critical points will be considered individually later, no corrections for them are included in the measurements.

Foreshortening occurs only in certain chromosomes as indicated in figures 27 to 30 and 33 to 36. This is, however, in the cells represented by figures 27, 28, 33, and 34 so slight that the whole chromosome can be seen at one focus, the foreshortened part appearing only slightly hazy. In the cells represented by figures 29, 30, 35, and 36 it is a little more. Measurements with the fine-adjustment graduated wheel, made more accurate with a sharper pointer made of a pin, indicated this sagging to be not more than 2.5μ (one division of the fine adjustment wheel) in any case. Corrections[5] made for this foreshortening are

of the shortened portion. Double the length of this hypotenuse minus the orignal measurement of the shortened portion is 0.2 mm., which is the maximum amount of shortening due to the vertical sag of any twisted portion. This amount added to that caused by the lateral deviation made the total shortening in one twist amount to 0.4 mm. Although this determination cannot be considered entirely accurate, it is a close approximation.

[5] The correction was made as follows. The amount of vertical deviation was read from the fine-adjustment wheel when the objective was focused as nearly as could be judged upon the middle of the lowest and highest points of the foreshortened portions. All measurements for a given complex were made with the same part of the fine-adjustment screw, thus avoiding different pitches in the thread. The reading (2.5μ for each division) gave the actual differences of vertical positions. This multiplied by the magnification and divided by 1000 converted the figure into millimeters, the units made use of in the drawings. By using this distance as the altitude of a right triangle and the measured longitudinal extent of the foreshortened portion as the base of the triangle, the hypotenuse (which represented approximately the correct length of the foreshortened part) was determined. This was substituted for the original measurement of the foreshortened portion.

only approximate, because it is very difficult to determine accurately the amount of vertical deviation, and its longitudinal extent, as well as its exact course. In figures 27 to 30 and 33 to 36 corrected figures are used, and the amount included in each measurement for foreshortening is indicated.

There are two other conditions which do not give rise to actual errors in measurement, but do interfere with precision of results and may well be considered here. 1) A possible unequal contraction of chromosomes. Wenrich ('16) observes that chromosomes A and B condense before the other chromosomes in the spermatogonia and tetrads of Phrynotettix, and ('17) he shows that one homologue of chromosome 4, cell E, plate 2, contracts more rapidly than the other. 2) Since so many of the chromosomes of Ambystoma are so long and composed of two intertwined chromatids, there is considerable possibility of a stretching due to bending and other stresses still present in the complexes nearing the metaphase. As Meves ('11, p. 247) points out, under these conditions two chromosomes could be of different length and of equal volume. Even an imperceptible difference in diameter of parts or all of two chromosomes of equal volume might cause considerable difference in their lengths. This difference would of course be proportionally greater in the longer chromosomes so that measurements of the shorter chromosomes of a cell might strongly indicate the presence of pairs while the homologues of the longer pairs would show quite wide differences in length. A case of very perceptible stretching is to be seen in chomosomes '*s*,' figures 9 and 12.

Effects of technique. The differences which may arise in the chromosomes of different cells of even the same tissue due to different effects of fixatives, and all other effects of technique, do not affect relative measurements of chromosomes in the same cell. For it is extremely improbable that the lengths of chromosomes of the same cell which are so equally close to the surface of these membranes would not be similarly affected by the action of these various reagents and processes. It is also improbable that inherent differences among the homologues would cause a differential change of length under these conditions.

Summary. The combined instrumental and personal errors are reduced to practically zero by averaging several measurements made upon different drawings. The errors due to twisting of chromatids about one another are largely neutralized and are considered individually later. Therefore, except for possible unequal contraction and stretching of homologues, only the measurements of those chromosomes which are foreshortened contain appreciable errors. It is thought that these errors, after corrections have been made, probably do not in any case exceed 1 mm. The presence and amount of error due to unequal contraction and stretching in any particular chromosome is an uncertainty, but if existing would probably be greater in the longer chromosomes.

c. Results of measurements. 1. Criteria for determining pairs. Before considering the results of the measurements, it seems desirable to state what the criteria are that will demonstrate whether pairs (p. 185) are present among the chromosomes at these particular stages of mitosis. In the absence of definite minute morphological characteristics, such as a repeated occurrence of marked granules, constant in position and size, which Wenrich ('16) describes for certain Orthopteran chromosomes, the next most exact criterion for determining the presence of pairs in diploid cells would be a duplicate series of chromosomes of equal volume. But in these chromosomes trustworthy volumetric determinations cannot be obtained, for the above-mentioned intertwining of the chromatids and the stretching of the chromosomes would cause variations in diameter which could not be measured accurately, and these errors would be cubed in the volume. Consequently linear measurements, supported by form, have been chosen as giving more trustworthy data.

Upon this basis, in order to constitute undeniable evidence that the chromosomes form a duplex series, there are two conditions which should be met. First, when the chromosome lengths are plotted in a graph (e.g., figs. 33 to 37), they should definitely associate themselves in twos of equal lengths. Second, the differences in length between successive pairs, as indicated

by the first condition, should exceed the errors of measurement by a good margin. Unless the above conditions are met, the errors of measurement make it possible to contend that the chromosomes are arranged in a series of successively increasing lengths which bear no relation to one another and therefore do not represent pairs.

In addition to the above, the form of the chromosome, which is probably determined in large part by the position of the spindle fiber attachment, may be used as an aid in determining which chromosomes are homologues. McClung ('14, p. 674) pointed out that, although the spindle fiber attachment may be different on different chromosomes, nevertheless, for each chromosome "it is most precise and constant" in the individual. Carothers ('17, p. 470) has shown that for certain tetrads (e.g., figs. 32 and 63) the point of spindle fiber attachment on one homologue is different from that on the other. But she also finds that the point of fiber attachment is constant on a given homologue for each individual. She shows (figs. 32, 32a and 63, 63a) that the point of spindle fiber attachment on these homologues in the spermatogonia is preserved in the tetrads. However, an exception to constancy of fiber attachment in the individual has been noted by Wenrich ('16). He found in a rod-shaped tetrad of another genus that the fiber attachment might shift from one end of the chromosome to the other in certain individuals. Therefore, according to the theory of the individuality, in the somatic chromosomes the homologues of certain pairs of chromosomes may be expected to be unlike in form. However, individuals showing such conditions are very few and should be considered exceptions rather than the rule. There is the possibility that these heteromorphic homologues may not be confined to the Orthoptera, and certain cases in Ambystoma make this appear to be so.

Finally, it would be remarkable if any material satisfied the above criterion in all points. The small difference in length between some chromosomes makes it impossible to demonstrate beyond doubt the presence of pairs among them. Again, the possibility that the chromosomes may not condense at equal

rates, and that unequal stretching, especially between the longer chromosomes, may occur during mitosis, increases the difficulty in obtaining accurate metric comparisons, and may interfere with perfect certainty in the interpretation of the results. Furthermore, some variation is characteristic of living material and hence slight differences in relative length and form in different cells would be expected rather than absolute uniformity. McClung ('17, p. 567) finds in certain Orthoptera that the accessory chromosome, although unmistakably distinguished from the euchromosomes, is not always of the same relative length in different individuals of a given species, for it sometimes occupies the fourth and sometimes the fifth position in the series of lengths. However, it must be remembered that this is the sex chromosome which at the metaphase (the stage of greatest condensation of the euchromosomes) is already becoming diffuse. Differences in the degree of condensation might therefore be involved in the differences of relative lengths. And again, since individuals vary in their morphological characteristics, why should it be expected that the chromosomes of two different individuals should be of exactly the same relative lengths at the same stage of mitosis? In view of universal variability, homologous chromosomes, which are derived from different individuals and which may be expected to maintain their individuality, should not invariably be of exactly the same length. As discussed (p. 217), the observations on different Orthoptera by several authors indicates this to be so.

On account of these interfering factors it cannot be expected that homologous chromosomes will always be of exactly the same length at any particular stage of mitosis. Therefore, length and form, considered in a limited number of cells, from different individuals, cannot be regarded as conclusive evidence for or against the presence of chromosome pairs. Much more conclusive evidence would be had in a comparison of several somatic complexes from a single individual and with those of other individuals, a comparison of these with the diploid and haploid chromosomes of the germinal line, and a comparison of the complexes of parents and progeny.

However, although the measurements may not meet the above criteria in all chromosomes, there are certain cases which do meet them definitely, and strongly evidence the existence of pairs. This fact, together with the above consideration, makes it possible that all the chromosomes are in pairs.

Furthermore, it may be mentioned here that conditions which do not meet the above criteria fall far short of proving that pairs do not exist. The possibility still remains that two or more pairs may be of equal or nearly equal length. Such a condition is known to exist in certain Orthopteran chromosome pairs (Carothers, '17, pl. 1, tetrads 7 and 8) where the chromosomes are unquestionably known to be paired.

2. Evidence for the existence of pairs. On plate 9, figures 33 to 37, are five rows of vertical lines representing the relative lengths of the chromosomes of as many cells. The differences in the lengths of these lines and also the space between adjacent lines represent relative differences in chromosome length. For convenience the lines are made twice the length of the chromosomes as drawn and the width of the spaces are made eight times the difference in length. The lengths of the chromosomes, the amounts included for foreshortening, and the form of chromosomes for each of these cells are also shown, respectively, in figures 27 to 31 and 33 to 37.

A part of the evidence which these graphs present for the existence of pairs is three outstanding characteristics common to all of them. First, there is a graded series of chromosome lengths from the shortest to the longest; second, there is a marked sameness in the relative chromosome lengths of these cells which appears in the approximately constant presence of groups containing the same chromosome pairs, and, third, a similarity of form between homologues.

The pairs, in accordance with the above criteria, were determined primarily on the basis of chromosome length, supported by a comparison of form. The graphs and figures of each complex measured show that certain chromosomes are very probably homologues. In other cases a number of chromosomes are so nearly of the same length that, according to the criteria, the

homologues of the pairs cannot be determined with certainty, but they doubtless exist. Since the measurements of figures 33 and 34 are very nearly accurate and present the most reliable evidence, these will be considered separately from the others. For convenience the pairs may be considered in two groups, the first including those which differ greatly in length from their neighbors (pairs 1, 2, and 8) and the second including the remainder in which the pairs are not so clearly distinguished.

It is all but certain that the chromosomes represented in each of pairs 1, 2, and 8 in both of these figures are homologues, for they almost completely satisfy the criteria outlined above. In these pairs there is foreshortening in only one chromosome and in pair 8 the error of 0.8 mm. in both complexes due to twisting of the chromatids is negligible. The homologues are of approximately equal length, and the difference between each pair and the adjacent pairs is so much greater than the error of measurement that it is improbable that the condition represented by these three pairs in both complexes is merely a matter of chance. Furthermore, there is a close resemblance of form between these homologues. A comparison of other cells of the same individual, if available, would be expected to show that this condition is constant in all the cells as is shown by comparable cases of constancy in the germ cells of individuals of certain Orthoptera (p. 219). It can, therefore, be maintained with considerable confidence that these particular chromosomes of equal length and sameness of form actually constitute pairs. The measurements of the chromosomes of these pairs in other cells as discussed below give similar although less conclusive evidence.

Among the chromosomes of the second group in these two cells there is strong evidence for the existence of pairs, but the small difference in length and the errors due to twisting and possible stretching make it inconclusive. In figure 33 the homologues as shown in each of pairs 3 to 7 are so nearly of the same length and form (fig. 27) that one may believe that they constitute pairs as represented. Pair 3, in addition, is well separated from those adjacent. Although pairs 4 to 7 appear to be actual pairs, the chromosomes of this series differ so little in length that

the criteria adopted are not entirely satisfied. There is a chance of doubt, therefore, of the validity of the pairs as indicated.

As mentioned above, it will be noted that the groups into which the chromosomes of this cell are associated are repeated in the other cells. The similarity of grouping is very marked, especially in the formation of two large and distinctly separated groups, one containing pairs 3 to 7 and the other pairs 9 to 14. In pairs 3 to 7 of figure 34 the condition present in figure 33 is duplicated, except that pair 3 is not so well separated from pair 4, due to the fact that both homologues of pair 4 are relatively shorter in the former complex. Concerning the homologues of pair 6 there is some doubt. I have interpreted the end of chromosome 30 + (fig. 28) as bending back upon the main portion of the chromosome, and have estimated the length of this portion.

Of the remaining six pairs (9 to 14) in both cells several are quite clear, but on the whole the possibilities of error and the differences in length between successive pairs is too small to satisfy the second criterion fully. In figure 33, on account of the practical absence of twisting in pair 12 and adjacent pairs, the condition for determining pairs is very closely satisfied. The chromosomes of pair 9 were considered homologues through a process of elimination. They differ 12.4 mm. in length, but agree in form (fig. 27). This condition will be discussed later (p. 217).

In figure 34, pairs 9, 11, and 13, although not sufficiently separated to constitute an unquestionable demonstration of pairs, are fairly well separated and the homologues of each pair, after allowance is made for errors due to twisting, are of nearly equal length. One homologue of pair 10 (fig. 28) is imperfect. Pair 14 is well separated in the graph from pair 13. Approximate corrections made for the kink in the shorter member and for the slight foreshortening at that point make its length approximately the same as that of the longer member. The homologues of all these pairs agree very well in form (fig. 28), in spite of the fact that some may not yet have assumed their final shape.

The measurements represented in figure 35 are nearly as reliable as those of the preceding cells. The amounts included for very slight foreshortenings are indicated in figure 29. The relative chromosome lengths form approximately the same groups as those of the other cells, and the evidence for the existence of pairs is strong. As contrasted with figures 33 and 34, the chromosomes of pairs 1 and 2, when approximately corrected for foreshortening, do not entirely meet the conditions of the criteria, but their lengths and form strongly support the probability that they are homologues. The chromosomes of pairs 7, 8, and 10 differ somewhat in length. They do not appear foreshortened, and although possibly present there is no perceptible stretching in them. These differences may be due to an unequalness of homologues as discussed later (p. 217). On the other hand, the chromosomes of pairs 3 to 6, 9 and 11 to 13 are, except as noted in figure 29, unforeshortened and of equal length, they agree in form and present strong evidence for the existence of pairs. The greater part of the difference in length between the homologues of pair 14 is due to stretching.

The measurement of the chromosomes of the cell represented in figures 30 and 36 are somewhat less favorable for measurement than those of the preceding cells, because in addition to a moderate amount of twisting there is slight foreshortening in many of them. Although the lengths have been approximately corrected for this foreshortening (figs. 30 and 36) the measurements cannot be considered so accurate and reliable as those of figures 33 and 34. The relative lengths of the pairs closely parallels that of the preceding figures which results in a similar distribution in the series. As contrasted with figures 33 and 34, the chromosomes of pairs 1 and 2 fail to satisfy the criteria, and this is apparently not due to errors in measurement. The large difference of 5.6 mm. between the homologues of pair 7 recalls a similar difference in pair 9 of cell 33. Pairs 8 and 9 clearly satisfy the conditions of the criteria and the remainder of the pairs duplicate the conditions in figures 33 and 34.

The chromosomes of the cell represented by figures 24, 31, and 37 are foreshortened in nearly every case and were measured

only in order to learn whether they constitute a series which would indicate that they belong to one cell. Only one set of measurements was made. Consequently, the figures are not so accurate as those of the other cells. Pairs 1 and 2 are readily recognized because they are well separated from each other and adjacent pairs. Pair 8 which stood out clearly in figures 33, 34, and 36, occupies a similar position here, but its homologues according to these less correct measurements differ about 4 mm. in length. The relative positions of the pairs practically duplicate those of the other cells. I have not attempted to make corrections for foreshortenings, but as nearly as I can judge, the chromosomes of pair 1, if corrected for foreshortening, would differ in length a little more, homologues of pair 2 would differ less in length, and the homologues of pair 8 are foreshortened about equally. Approximately the same condition exists in the other pairs, so that the matching as indicated would not be disturbed sufficiently to alter the grouping of the pairs. In this cell chromosomes of pair 12 differ by approximately 10 mm., which recalls a second time the condition in pair 9 of figure 33.

Further consideration of the form of the chromosomes in all these figures furnishes additional strong evidence that the chromatin is definitely organized. As indicated in anaphases, the general form of the chromosomes in the metaphase of these somatic mitoses is determined by the point of spindle fiber attachment. The complexes represented in figures 27 to 32 are early metaphases, and the final form which the chromosomes will take is quite apparent, although in some cases (e.g., pair 8, fig. 27) it is not entirely clear.

In all of these figures, as previously mentioned, the form of the homologues of each pair is practically the same, even in cases where the final form has not been reached. Only three pairs (5, fig. 29; 7, fig. 30; 5, fig. 31) are exceptions, and this may be expected as indicated by a like condition in the heteromorphic pairs of certain Orthoptera (Carothers, '17). A comparison of several complexes from the same individual would probably show this condition constant for the individual as in the Orthoptera. A further comparison of each pair in any figure with

the corresponding pairs of all the other figures also shows a striking correspondence of form in these chromosomes. The homologues of the unusual cells represented by figures 31 and 32 may not be correctly determined, as previously mentioned.

Such agreement and constancy of form between homologues and between corresponding pairs of different individuals cannot well be considered as due to chance and indicates a definite organization of the chromatin.

3. Summary. The strongest evidence for the existence of pairs is the fact that the chromosomes indicated as pairs 1, 2, and 8 in figures 33 and 34 completely satisfy the criteria. Although these pairs fail to do so in figures 35 and 36, they are recognizable. Among those chromosomes composing the two large groups in which the chromosomes differ so little in length (pairs 3 to 7 and 9 to 14) the evidence presented by the lengths of the chromosomes does not conclusively demonstrate nor deny the existence of pairs because of the various factors inherent in the nature of the material. However, as represented in the graphs and figures, the lengths and forms of these chromosomes strongly indicate such a duplexity. The cases in which the chromosomes of a pair differ somewhat in length do not constitute contrary evidence since homologues are not always of equal length as explained on page 217. Furthermore, the constancy of chromosome number, the presence in all the cells of certain groups composed of the same number of chromosomes with approximately the same relative lengths is further strong evidence of a constancy of chromatin organization and that the lengths of the chromosomes are not due merely to chance.

It seems to me to be a very difficult task to demonstrate conclusively by means of measurements the existence of pairs in these and similar somatic complexes. To furnish anything more than strong supporting evidence is almost impossible because of the various difficulties inherent in the nature of the material and because of the fact that homologues, as shown in exceptional cases, are not always of equal length, a fact which has been actually observed in the germ cells (tetrads) of certain Orthoptera by several authors. The same conditions make it

just as difficult to demonstrate the absence of pairs. It seems to me that the evidence in the Dipteran somatic complexes, where the members of a pair lie parallel and adjacent to one another, together with the already large and well-supported evidence of pairs in the various generations of the germ cells throw the balance greatly in favor of the presence of homologues.

DISCUSSION

A. Introductory statement

The foregoing observations upon the constancy of chromosome number and the existence of pairs in the somatic chromosome complexes have their chief importance in their relation to the Roux-Weismann hypothesis that the chromatin is the idioplasm, which is differentially organized and linearly arranged, and that this organization is perpetuated. This hypothesis received important support from the theory of the individuality of the chromosomes as set forth by Van Beneden ('83) and strongly maintained by Rabl ('85), Boveri ('88, '02), and numerous other more recent investigators. The morphological evidence accepted as supporting this proposition is an essential constancy of number, size, form, and behavior.

Since McClung ('17) has so recently thoroughly considered the theory of individuality, this discussion is confined to the particular phases of the supporting evidence which are directly related to the observations made upon this material. These phases are essential constancy of number, of size, and of form.

B. Constancy of chromosome number

Della Valle has strongly attacked this theory on the basis of inconstancy of chromosome number. He arrives at the conclusion ('09, p. 120 ff.) that the number of the chromosomes is the quotient of the quantity of the chromatin divided by the average size of the chromosomes; that their size is variable according to the nature of the elements and the conditions in which they are found, and ('11, p. 188) that the size and number

of the chromatic elements are directly comparable to the size and number of the fluid crystals which are formed in a solution under different conditions.

These contentions he supports with the claim ('09) of a variation of nineteen to twenty-seven chromosomes in forty mitoses of the peritoneum, and ('11) by a very large variation in the blood cells of Salamandra maculosa. These observations he supplements with a long list of citations of chromosome numbers which he interprets as supporting his contention.

But, following an apparently frank and critical discussion of the accuracy of his observations in the peritoneal cells, he says ('09, p. 116) that he is only sure of his enumeration in twenty-five of the forty cells discussed. These twenty-five complexes he describes as being very clear. The range of variation in these cells is as follows:

Number of chromosomes....	19	21	22	23	24	25	26	27	
Number of mitoses.....	1	1	1	6	16	12	2	1	total 40
Number of mitoses.....		1	1	3	10	8	2		total 25

An examination of his descriptions and figures may indicate to some extent the reliability of these counts.

His count of 22 chromosomes was made upon a polar view of a very late anaphase (fig. 2) in which he states the smaller chromosomes in the center of the complex were beginning to go to pieces and becoming indistinct, and his only doubt is whether the chromosome numbered 18 is one or two chromosomes. But, judging from similar stages in my material, it seems to me that where the chromosomes are beginning to go to pieces in as crowded a condition as this must be, such a complex is not a safe object for an exact chromosome count.

Instances of this kind make it seem possible that conditions which he considers clear for an exact count might be much less conclusively clear to others, and that his drawings do not represent the actual conditions in his complexes.

Since his citations of chromosome number variations found in the literature have been discussed by Montgomery ('10), Wilson ('10), and by McClung ('17, p. 548 ff.), it is not necessary to

review them extensively. But Della Valle's attitude and his conception of what constitutes clearness and certainty may be better understood in the light of some of these citations of chromosome variations, especially in the Amphibia, which he presents as valid evidence of variation in chromosome number. He quotes ('09, p. 35) Flemming ('81, ['82] p. 51) and Rabl ('84, ['85] p. 248 to 250) as reporting variations of from seventeen to twenty-four in the gill epithelium of Salamandra maculosa. Flemming explicitly states (pp. 51 and 52) that in the three cells which admit an exact count there are twenty-four chromosomes, that in about twenty other cells he counted from seventeen to twenty-four, but was not certain of the number, and assumed that there were twenty-four. Rabl says ('85, p. 248) that up to that time only eleven unquestionable counts had been made and each of them showed twenty-four chromosomes. In no exact counts in any cell had a different number been found. Della Valle seems to think that Török's ('88) figures of erythrocytes of Salamandra maculosa show a variation. This work was not concerned with chromosome number and the figures were not intended to show the number of chromosomes in the cell. His citations of the work of Carnoy and Lebrun ('00) on Rana temporaria, and of Lebrun ('02) on Diemyctylus and Bombinator may be criticised because the authors were primarily concerned with other considerations and only gave approximate number determinations. Winiwarter ('00, p. 699), as cited by Della Valle, reports a variation of chromosome number in the rabbit; but he states that he is uncertain of his counts. The variations reported by Barratt ('07, p. 376), in proliferating epithelium of the rabbit are in pathological tissue, and, moreover, his counts are uncertain. Montgomery ('10) has shown that many other such citations are misinterpreted.

The above cases represent Della Valle's inexact and uncritical attitude in relation to data that seem to serve his purpose, and this creates the suspicion that his attitude interfered with the accuracy of his observations when he counted the chromosomes in his own material. This suspicion approaches a probability in view of the fact that numerous competent investigators who

have made extended and careful studies of the germinal and somatic mitoses of the same species mention no variation in chromosome number. Furthermore, Meves ('11), who attacks the theory of individuality, fails to substantiate Della Valle's observations, and it is not at all likely that he would have failed to mention any variation observed. He appears to believe (p. 296) that the number is constant. However, Heidenhain ('07, p. 176, figs. 80 and 81) shows a polar view of a late prophase and a lateral view of a metaphase with twenty-six and twenty-two chromosomes, respectively, and states that such irregularities *occasionally* occur. An occasional variation is not surprising, but variations as numerous as Della Valle claims to be present are unusual. Flemming ('90, p. 78) states that he observes in the lungs of Salamandra maculosa numerous atypical mitoses with very short chromosomes. He gives no further discussion and no figures to indicate what kind of cells they are nor whether they are normal. In the ten cells of the lung of Ambystoma tigrinum (table, p. 177) there were no variations in chromosome number, and with the exception shown in figure 26 I observed no abnormalities. Della Valle's ('11) figures of blood cells in Salamandra maculosa, which he claims show an extreme variation in chromosome number, appear very much like cells undergoing disintegration.

To the above evidence of the questionableness of Della Valle's results may be added the results of the sixty-six counts in Ambystoma tigrinum showing no variation in number. The important fact that these counts were made with extreme care (p. 177) in the somatic cells of the same and other tissues of a closely related species, and made in uncut membranes (which Della Valle emphasizes as important for accurate counts), further strengthens the already strong probability that his number determinations are incorrect.

There are certain characteristics in his figures that also indicate that his drawings are none too accurate. He notices that the chromosomes are twisted, but he does not show what constitutes the twist. That the peritoneal chromosomes of Salamandra maculosa are each composed of two separate chromatids twisted

about one another is plainly evident in Meves' ('11) figures 11 to 15 which show each chromosome to be of variable width. These figures are exactly comparable to my figures 1 to 8, and 9 to 23 which demonstrate that this variation in width is due to the twisting of the chromatids. Della Valle represents each chromosome to be of uniform width excepting an occasional split in the end of some chromosomes. If he does not see chromatids in any of the chromosomes which he has drawn, either his observations, his technique, or both are faulty. Furthermore, the above evidence together with his attitude make it uncertain whether his preparations were as clear or the chromosomes as distinctly separated from one another as his drawings indicate.

Finally, Della Valle's above demonstrated attitude, the absence of confirmatory evidence for his contentions, his questionable ability as an observer as indicated by his drawings, and the results of critical counts in Ambystoma tigrinum, all support the view that his observations and conclusions are incorrect.

But upon the assumption that they may be partially correct, there are some possible explanations for the presence of variation in the peritoneum of Salamandra maculosa. 1) One or more chromosomes of a complex easily could have been disturbed, as is evident from my figures 22 and 24 to 26. This could account for number deficiencies and perhaps also for excesses. 2) Champi ('13, p. 181) claims that chromosome number can vary by fragmentation under the influence of certain external stimuli. Della Valle ('09, p. 86) says the number of mitoses can be increased by keeping the larvae covered with a blue glass. If Della Valle did this, and if such a stimulus could produce fragmentation, a bare possibility is offered for a disturbance of chromosome number. 3) There is also a slight possibility that the larvae had been kept in captivity and might in consequence have been sufficiently pathological to produce abnormal mitoses. 4) In an investigation on certain Orthoptera now in progress in this laboratory, Mr. Carroll observes that in three individuals some of the few dividing spermatogonial cells contain, in addition to the normal number of twenty-three, one, and some two

extra chromosomes (dyads). This is a small variation in chromosome number in the individual of from twenty-three to twenty-five. In each of four individuals, including the above three, he finds that in from one to three of the primary spermatocytes observed in division, one of the eleven tetrads normally present is replaced by two separate dyads. Both of these dyads in the division of the cell may pass undivided to either daughter cell with or without the accessory chromosome. One of the secondary spermatocytes resulting from the division of the cell in which these two dyads accompany the accessory chromosomes receives twelve dyads plus the accessory and the other receives ten dyads. The other spermatocyte in which two dyads do not accompany the accessory chromosome gives rise to one secondary spermatocyte with ten dyads plus the accessory, and another with twelve dyads. This would make possible four classes of spermatozoa containing ten to thirteen chromosomes.

If, similarly, in Salamandra maculosa one of the twelve tetrads normally present in the spermatocytes and in the oocytes should be replaced by two dyads, there would be produced gametes with eleven, twelve, and thirteen chromosomes. These gametes would produce zygotes (individuals) having twenty-two and twenty-six chromosomes, respectively, a variation comparable to that claimed by Della Valle. Further, if extra chromosomes can thus appear in the germ cells of an occasional individual, the same might also occur in the somatic cells. But this variation should be expected in but few of the total individuals, making the proportion of cells containing the normal number greatly predominating. In Della Valle's counts about one-half contain the normal number which is far too small a portion unless such variation is much more common than is at present known.

Although the above is a clear case of a small variation in chromosome number *in the individual*, it must be clearly understood that these cases are exceptional and do not represent the normal condition. The chromosome number may vary in the species, but it is usually constant for the individual, as has been especially pointed out by Wilson ('09, '10), Carothers ('17),

McClung ('05, '07, and '17), and others. But it is very important to note that these irregular chromosomes arise and perpetuate themselves in a manner entirely consistent with a definitely organized chromatin and furnishes no support whatever for Della Valle's contention that the chromosomes are comparable to crystallizations of a salt solution and that their number in any cell is dependent upon the law of chance.

C. Variations in other Urodeles

Snook and Long ('14) find in the spermatogonial cells of Aneides lugubris nine containing clearly the usual number of twenty-eight chromosomes, and one cell with twenty-three. There are no other authentic reports of variable chromosome number in individuals of the Urodeles. The counts of Kölliker ('89), Fick ('93), and Jenkinson ('04) in the cleavage stages of Axolotl (Ambystoma tigrinum) were made for other purposes, and were not presented as accurate number determinations. Likewise, the counts of about eighteen to twenty-four, Champi ('13, p. 124) in several other Salamanders are only approximate.

D. Variations in other forms

Since a somewhat extensive tabulation of comparative germinal and somatic counts has been made and discussed by Hoy ('16), Harvey ('16), and briefly reviewed by Hance ('17 b), a repetition of this discussion is of little value. However, in the review of reported cases of variation a few general considerations have impressed me as worthy of mention. These may seem commonplace, but are evidently not altogether realized by some who are none too critical in their discussion of the significance of these enumerations. 1) As Montgomery ('01) long ago suggested, it is important to distinguish between variation in the chromosomes in the germinal line and those of differentiating somatic cells. In the germ cells I believe it can be stated safely that there are no certainly demonstrated variations in number which do not conform to a definite organization of chromatin. From time to time cases of apparent variation have appeared

and again disappeared when thoroughly understood (e.g., Metapodius Wilson, '10, and the sex group of Ascaris lumbricoides Edwards, '10, and multiple chromosomes of certain Orthoptera Woolsey, '15, Robertson, '16, and McClung, '05, and '17). 2) In considering the significance of variations, it should be remembered that there are normal and abnormal conditions (p. 202). 3) Metz ('16), Hance ('17 a, b), and Whiting ('17) have called attention to the necessity of proper technique. This is not always an easy matter to judge, especially in absence of material for comparison. 4) In determining the presence or absence of variation in any material, a very rigid line should be drawn between accurate enumerations and those involving varying degrees of interpretation, e.g., Winiwarter's ('00) cited variations in the amnion and omentum of rabbit embryos were uncertain and interpreted. 5) Finally, observers should maintain an exacting standard in distinguishing between that which is considered 'certain' and that which is interpreted. This is especially true in counting small chromosomes.

E. Fragmentation

Hance ('17 b, '18 a) found the spermatogonial number in the pig, and the diploid pollen-mother cells in Oenothera scintillans to be constantly forty and fifteen, respectively. But the somatic chromosomes vary from forty to fifty-seven in the pig and from fifteen to twenty-one in Oenothera scintillans. He presented metrical evidence that this variation is due to a fragmentation, probably of the longer chromosomes. He maintains that these fragments divide normally with the other chromosomes, and that therefore this fragmentation does not oppose the theory of the individuality of the chromosomes.

However, the probability that this variation in Oenothera is much less, and that most if not all of these fragmentations are invisibly connected with the main part of the chromosome is strongly supported by the conditions in Ambystoma material and by Hance's ('18 b) later observations upon additional Oenothera material from the same source. Both he ('17 b, p. 90)

and Hoy ('16, p. 356) review other cases of fragmentation in Ascaris megalocephala (Boveri, '99, '04), Angiostomum (Schleip, '11) and Fragmatobia (Seiler, '13).

In Ambystoma tigrinum (p. 179) and in Salamandra maculosa (Della Valle, '09, fig. 11), and as stated above, in Oenothera, the fragmented portions are directly in line with the main portion of the chromosome. This may be due to the absence of chromatin on the linin or failure of the chromatin to stain at that point. The fact that in several cases (e.g., *f*, figs. 5, 6 and 7) the space between the fragment and the main portion of the chromosome was uniformly faintly stained lends support to the suggestion. Other cases exhibited connections consisting of various amounts of strongly stained chromatin (e.g., *chr.f.*, figs. 5, 14, 15, and 21). All mitoses in the gill plates (the most in prophases, fig. 5) showed the largest number of instances of this condition; the peritoneum contained scarcely any. This might be explained as an effect of inferior fixation (p. 173) were it not for the fact that a considerable amount of apparent fragmentation is present, even in the metaphases of the tail epithelium which are fixed under the most favorable conditions. The reason for this is not clear.

F. The existence of pairs

The question whether the chromosomes exist in a duplicate series is significant in two respects: 1) in its relation to the mechanism of heredity as suggested by Janssen's chiasmatype theory and by the brilliant work of Morgan and his co-workers; 2) as a further index of the constancy of the organization of the chromatin. This constancy is vitally related to the theory of the individuality of chromosomes.

It will be convenient to consider separately the evidence of the existence of pairs in the germ cells and in the somatic cells.

a. Pairs in germ cells. Van Beneden's ('83) hypothesis, that one-half of the chromosomes of an individual are of maternal origin and that the other half are of paternal origin, has been verified in many cases. That this double set of chromosomes

exists in pairs in the germinal line is evidenced by their behavior during the maturation period.

Montgomery ('01) presented evidence for the recognition of pairs in the spermatogonia, basing his argument upon the significance of the chromosome number in Euschistus. Sutton ('02) showed by means of a comparison of many camera-lucida drawings of spermatogonial complexes of Brachystola magna that these chromosomes form a duplicate series of lengths, and by means of measurements with a pair of dividers that the chromosomes of the early primary spermatocyte prophases are graded into the same series of relative sizes. Meves ('11) interpreted his measurements upon spermatogonia of Salamandra maculosa as failing to demonstrate pairs. Meek ('12) has made linear measurements upon the spermatogonia and secondary spermatocytes of a number of animals, interpreting his results as confirming the claim of the existence of pairs. Robertson ('15, '16) also supports this view with metrical data in certain Orthoptera, and Hance ('17 b; '18 a) confirmatively interprets his measurements in the germinal and somatic cells of Oenothera and the pig. In unmeasured spermatogonial chromosomes of the Diptera, Stevens ('08, '10, '11), Metz ('14, '16), and Whiting ('17) show very convincing evidence of pairs for the homologues are associated side by side. Wilson's ('06, p. 11) figures of Anasa and Hoy's ('16, p. 336; '18) figures of Anasa, Epilachna, and Diabrotica also support this conclusion. The majority of other authors as a result of their general observations have expressed the belief that the chromosomes exist as a duplicate series.

Furthermore, the existence of pairs in the spermatogonia is practically proved by parasynapsis where the chromosomes of the last spermatogonial division unite side by side and remain so until separated by the reduction division. This statement is made possible by Wenrich ('16) who, besides confirming the already numerous and all but conclusive evidences of parasynapsis by Janssen ('05, '09), A. and K. E. Schreiner ('06, a and b; '08, a and b), Wilson ('12), and many others, carries the demonstration a step further by actually tracing a well-marked chromosome pair A (p. 76) continuously through every stage of

spermatogenesis from the early spermatogonia to the spermatids. He thus demonstrates that the conjugating elements are chromosomes and are morphologically identical with the spermatogonial chromosomes. That one of the homologues of each conjugated pair is maternal and the other paternal is very probable, as has been shown by the observations of Van Beneden ('83) and numerous later authors, especially Mulsow ('12).

It remains to be seen whether the pairs of maternal and paternal homologues present in the germ cells during the maturation period maintain their identity in the germinal line between the time of fertilization and the first observations upon the spermatogonia. This has been accomplished in part. Mulsow ('12) has followed the actual chromosomes of the living spermatozoon of a parasitic trematode, Ancyracanthus, into the egg, and has found the expected number of chromosomes in the two pronuclei and cleavage stages. He also observes that the chromosomes of the cleavage nuclei show in many cases a tendency to lie parallel to one another, and suggests that this is an approximation of maternal and paternal chromosomes. Boveri ('87, '92) traced the chromosomes of the primordial germ cell of Ascaris univalens through the cleavages from the two-celled stage, and Moenkhaus ('04), Morris ('14), Richards ('17) have traced the persistence of individual chromosomes through several cleavages of hybrid eggs of Fundulus. If this persistence of the chromosomes is permanently maintained, the observations of the above authors make it probable that the maternal and paternal chromosomes form a duplicate series throughout the germinal line.

b. Pairs in somatic cells. Since the existence of chromosome pairs can be considered to be all but proved throughout the germinal line, it remains to be seen whether or not the chromosomes of the somatic cells, which are really descendants of those of the germinal line, still retain this duplicate series and thus give evidence of maintaining their individuality.

The earliest observations bearing upon this question were made on Salamandra maculosa by Flemming ('82) and Rabl ('85), who observed that the chromosome segments were not of

equal lengths in the early spireme and metaphase stages. The general observations of many later authors, especially those studying Dipteran somatic cells (Metz, '14, '16 a, b; Hance, '17; Holt, '17; Whiting, '17) indicate these chromosomes to be paired. Observations of pairs in a large number of animals and plants have been extensively reviewed by Metz ('16, p. 245).

But no cases are recorded in which an attempt was made to determine accurately by measurement what relation the lengths of somatic chromosomes bore to one another until the work of Meves ('11). As a result of the discussion centering around the observations of Montgomery ('01) and Sutton ('02), he was led to attempt measurements in an effort to obtain more definite data, as suggested by Della Valle ('09, p. 109). He measured both spermatogonial and somatic chromosomes of various tissues in Salamandra maculosa. Della Valle ('12) made further measurements upon the same form, and agrees with Meves that their results do not confirm the observations of Montgomery and Sutton. Hance ('17 and '18 a) interprets his measurements upon the somatic chromosomes of Oenothera scintillans and the pig as confirmatory.

1. Meves' results. Since Meves has (p. 282) failed to confirm the results of other authors, it is desirable to reconsider his data in comparison with linear measurements upon cells of the same nature in the same kind of preparations and of the same tissues of another salamander, Ambystoma tigrinum, in an effort to form a judgment of the validity of his conclusions. For this purpose it is necessary to recall what criteria are required (p. 191) definitely to affirm or to deny the existence of pairs and under what conditions these criteria were satisfied.

The following conditions under which Meves' measurements were made allow the introduction of such a varying amount of error that the conclusions drawn from his results are of questionable value.

Instrumental and personal errors. Meves made his measurements evidently upon a single drawing, probably somewhat carefully executed, which means, according to a series of tests in my own attempts to be accurate, that he has a minimum instru-

mental and personal error of about 0.6 mm. at his magnification. This, of course, is negligible in comparison with the large errors arising from foreshortening in numerous chromosomes.

Concerning the favorableness of the spermatogonial chromosomes for measurement, Meves says (p. 274) that by no means do all of the chromosomes lie in the equatorial plane; without exception the bend lies in the plane while the ends lie outside; in the drawings such chromosomes seem shortened and therefore the measurements upon these chromosomes would give only an approximate value. Judging from these statements and from the magnitude of error due to the slight foreshortenings in my material, his measurements very likely contain errors which amount to as much as 4 or 5 mm.

For measurements of somatic chromosomes he chose (p. 280) polar views of the transformation stages between the prophase and metaphase stages in the epithelium of the gill plates (figs. 16 to 18) and extraordinarily well-flattened polar views of prophases (figs. 11 to 13) and metaphases (figs. 14 to 15) in the peritoneum. In the three prophases of the peritoneum the chromosomes lay nearly or entirely parallel with the upper surface of the cell. According to this description, it is evident that these three prophases are the most favorable cells, and even in these the chromosomes are not entirely free from foreshortening. The chromosomes of the other cells probably were more foreshortened. Therefore, judging from results in Ambystoma, his measurements contain errors due to foreshortening which probably vary from 2 to 5 mm.

The amount of the errors which are due to the twisting of the chromatids of these chromosomes is uncertain. Such twisting is evidently present, as indicated by the irregular contour of his chromosome drawings which are similar to those of my own. The errors due to this twisting may largely neutralize each other as explained above (p. 189). He mentions also the possibility of different rates of contraction of the chromosomes, especially in the earlier stages. Having before us the conditions under which Meves made his measurements, we are in a position to judge their value more or less correctly.

As Meves states (p. 276), the differences in length between the spermatogonial chromosomes are too small, and the possible errors too great to affirm that these chromosomes are present in pairs of equal length. On the other hand, it should be added that these conditions offer no evidence against this claim. Furthermore, he shows a practically constant difference in length of 6 half-millimeters between chromosomes 8 and 9 in his table of measurements. An examination of these figures shows unmistakable evidence that there is foreshortening in chromosome 9 in three of these cells, and a probability that chromosome 8 is much less, if any, foreshortened than chromosome 9. There results, therefore, in all of these cells a relatively uniform distribution of the chromosomes into two constant groups, a point which supports the claim that the relative lengths of these chromosomes remain constant.

Concerning the somatic measurements, Meves concludes (p. 282) "Die Fragen, ob bei gewöhnlichen Gewebszellen des Salamandra Chromosomenpaaren unterschieden werden können, ist bereits von C. Rabl ('06, S. 72) verneint worden, ich muss mich ihm auf Grund der mitgeteilten Zahlen anschliessen" and "Die erhebliche Längendifferenz zwischen chromosomen VIII und IX, welche wir bei den Spermatogonien festgestellt haben, besteht bei den gezeichneten somatische Zellen nur in der Hälfte der Fälle." This means, of course, that he believes that the chromosomes are not distributed into two constant groups in each cell and that therefore the evidence of the constant organization of the chromatin is lacking in this respect.

As already stated, the possibilities of error are so great that nothing is conclusively affirmed or denied by his measurements. However, an examination of the drawings of the chromosomes in his figures indicates certain probabilities.

1. In three of the four cells (his figs. 15 to 17) in which the marked difference between chromosome 8 and 9 is not present, chromosome 9 is conspicuously foreshortened, and is in reality longer than his measurements indicate. Furthermore, chromosome 8 in these cells may or may not be foreshortened, at any rate it is probably much less foreshortened than chromosome 9.

A conservative estimate of foreshortening upon chromosome 9 in these cells, based upon computation of foreshortening in my material, amounts to a minimum of 2 mm., which restores in these cells the difference in length between chromosomes 8 and 9 found in the spermatogonial and the other somatic cells. These same chromosomes in the remaining cell (fig. 13) which lack this difference do not appear foreshortened, but owing to the various sources of error pointed out in the preceding pages, it is entirely possible that such a difference may also be present in this cell. These considerations make it probable that the difference between chromosomes 8 and 9 is present in eleven and possibly in all of the twelve somatic cells which Meves measured. It is beyond expectation that this difference should be exactly the same in every cell whether of the same or of different stages of mitosis.

2. Meves also observes a difference of 3 to 4 mm. between other chromosomes which theoretically should be homologues. In figure 11 between chromosomes 17 and 18 and between chromosomes 23 and 24; in figure 12, between chromosomes 11 and 12; in figure 13, between chromosomes 7 and 8, 15 and 16, 17 and 18, 23 and 24.

Attempts to apply corrections for the foreshortening evident in these cells, estimated upon the basis of his drawings as compared with similar cells in Ambystoma (figs. 5, 13, and 14), leave the situation about as it was. This is not surprising, considering the difficulty of judging the amount of foreshortening that is conspicuously present in some chromosomes and the uncertainty of its presence, or absence, in others. Meves' statement quoted above concerning the type of cells used for measurements makes it quite possible that a great many of these chromosomes are foreshortened in amounts varying from 2 to 5 mm. This opinion is strengthened by his generalized and unprecise statement concerning foreshortening and by comparisons with similar cells in Ambystoma.

The conspicuous foreshortening in some chromosomes, and the probability of it in others, seriously weakens the validity of his measurements. This is especially true of figures 11, 12,

and 13 showing late prophases in which there is usually much foreshortening and considerable inequality of homologues, as stated above. In five other cells (figs. 14 to 18) Meves' figures show but one case of a difference of 3 mm. and only five cases of a difference of as much as 2.5 mm. between homologues, as they are indicated by his measurements. But the various sources of error already mentioned make it uncertain as to what the actual lengths are.

To summarize the examination of the results of Meves' measurements, it may be said, 1) that the chromosomes of the spermatogonial cells fall into two groups, one containing chromosomes 1 to 8, the other chromosomes 9 to 24. 2) In the somatic cells, when corrections are made for evident foreshortenings, it is probable that in eleven of the twelve cells, and possibly also in the twelfth, the same grouping is present. This indicates a constancy of organization of the chromatin. 3) It is impossible either to demonstrate conclusively or to deny that these chromosomes are paired because, *a*) of the various sources of error present and, *b*) the small differences in length in the majority of cases between adjacent chromosomes.

2. Della Valle's measurements. Della Valle ('12) measured the lengths of chromosomes in the peritoneal cells of Salamandra maculosa shown in his ('09) figures 1 to 3, 8 to 9, and 12. The length of each chromosome was obtained by averaging two measurements made with a curvimeter upon a single camera-lucida drawing. He also attempts to determine the degree of concordance between the measured lengths of each of these chromosomes and the dimensions which would exist if the lengths of these chromosomes were determined by the laws of fluctuating variation. These latter figures he obtains by calculation from a table of figures compiled by Sheppard and published by Galton ('07). He interprets his data as demonstrating, 1) that the chromosomes of Salamandra maculosa do not exist in pairs; 2) that there is no constant grouping of chromosomes, such as is evident in the measurements of Meves and myself, and, 3) that the chromosomes are a series of variants subject to the laws of fluctuating variation as shown by the comparison,

given in his tables and curves, between the measured lengths of the chromosomes and the computed lengths that would be expected if the chromosomes were such a series of variants.

I believe Della Valle's conclusions are incorrect for the folowing reasons: 1) He fails to demonstrate the presence of chromosome pairs because, *a*) as discussed on page 201, his chromosome enumerations are probably incorrect and therefore his measurements do not represent the actual conditions; *b*) his measurements probably contain numerous errors of varying magnitude due to foreshortening (as well as to errors arising from measurements upon single drawings) even though he chose for measurements strongly flattened cells (p. 126); *c*) the differences in the lengths of these chromosomes are so small and the errors so great that it is impossible either to demonstrate or to deny a presence of pairs. 2) Failure to find a constant grouping among the chromosomes would result from the causes given in (a) and (b). 3) His interpretation that the chromosome lengths are controlled by the law of fluctuating variations is untenable because, even if his measurements were reliable and whether pairs do or do not exist, the differences in length between the chromosomes of Salamandra maculosa are so small that the degree of correspondence between their measured lengths and the calculated lengths of a series of variants, corresponding respectively to each of these chromosomes, would be fully as close as those which he presents in his tables and curves containing numerous and large differences.

3. Results in Ambystoma tigrinum. The chromosomes of Ambystoma tigrinum, fortunately, are more favorable subjects for measurements than those of Salamandra maculosa, because the relative differences in length between many pairs is so large that certain pairs and certain groups of pairs stand out conspicuously. The evidence presented in figures 33 and 34 is free from all errors of measurement except those due to twisting of the chromatids and to minute foreshortenings at non-critical points in the series. These errors have been approximately eliminated (p. 188) and do not seriously disturb the critical evidence of the chromosome pairs which are much shorter or longer

than adjacent pairs. The evidence in the other figures is but little inferior to that of figures 33 and 34. However, measurements of the chromosomes of so few cells are insufficient to furnish more than strongly supporting evidence of the existence of pairs, and of an approximate constancy of size relations between pairs in different individuals.

It may appear that these measurements support equally well Della Valle's claim that there are no chromosome pairs, but that the chromosomes form a series of variants. However, the consistent evidence of the presence of pairs among the shorter chromosomes, the possibility of unequal stretching of the longer homologues together with the known condition in Orthoptera that homologues of tetrads may be of unequal lengths lends greater support to the probability of the existence of homologues.

The following points need further consideration.

Large differences in length between homologues. The difference in length of 12.4 mm. between the homologues of pair 9 in figure 33 and the similar difference of 9.5 mm. between the homologues of pair 12 in figure 37, the smaller difference in pairs 7 and 8, figure 35, pair 7, figure 36, and pair 8, figure 37, may possibly be explained as follows:

1. Unequal homologues have been reported in Orthoptera by Baumgartner ('11), Hartmann ('13), and more thoroughly studied by Carothers ('13), Robertson ('15), and Wenrich ('16). The latter's observations are particularly significant. He found different conditions of inequality in two of the small tetrads of Phrynotettix. He designated these two tetrads as 'B' and 'C' and traced their history from the pachytene stages through the first maturation division. The homologues of tetrad 'B' were unequal in eleven of the thirteen individuals studied and were equal in the other two. Tetrad 'C' was found in three forms, designated as 'C_1, C_2, C_3.' 'C_1 is composed of very unequal elements, the larger of which possesses a relatively large terminal knob or granule which is not present on the other two. 'C_2' is a pair with equal members, each of which appears to be homologous to the smaller member of 'C_1.' 'C_3' is a pair of unequal elements, neither member of which appears to be

exactly homologous to the components of 'C_1' and 'C_2.' The smaller member resembles those of 'C_2' and may be homologous with them. It is important to note that the three last-mentioned authors find that each particular condition is constant for the individual in which it is found.

Although tetrads with unequal homologues among the longer chromosomes have not been observed in the Orthoptera, they might possibly exist in other animals. The above observations, especially those of Wenrich, offer a possible explanation for the inequalities between homologues observed in Ambystoma as well as in Salamandra maculosa. Furthermore, the condition found in tetrad 'C_2' may offer a parallel explanation for the different relative lengths shown in some cases between corresponding pairs in complexes of different individuals (e.g., pr. 4 and pr. 9). Of course much further data from both the somatic and germinal chromosomes is necessary before the above can amount to anything more than a suggestion.

2. Certain inequalities might be explained as due to the presence of a multiple chromosome similar to that which has been described by McClung ('05, '17), in Orthoptera, by Boveri ('09), Edwards ('10, '11), and Frolowa ('12), for Ascaris megalocephala, Boveri ('11) and Edwards ('11) for Ascaris felis, Stevens ('11) in Anopheles, and by King ('12) for Necturus. In these cases the sex chromosome has been interpreted as being attached to one of the euchromosomes, and thus there is present in the male an unequal pair of chromosomes which may parallel the condition in pair 9 of figure 33 and pair 12 of figure 37. In certain Orthoptera McClung ('17) finds that the accessory may be attached to different chromosomes in different individuals, which lends support to the possibility that this is the condition in Ambystoma. The presence of an X and a Y chromosome would also produce unequal homologues.

If either of these explanations be valid, such an unequal pair should appear in all the diploid complexes of approximately one-half of a somewhat large number of individuals, and similar conditions should be found in the maturation period. Unfortunately, the difficulty of obtaining a sufficient number of suitable

cells prevents extensive investigation of this point in the somatic cells for the present and makes this explanation only suggestive.

Constancy in the individual. It should be emphasized that the observations in the Orthoptera concerning the unequal tetrads (p. 217) and other heteromorphic tetrads (p. 192) strikingly demonstrate a constancy in the individual for the particular characteristic of each homologue concerned. In Ambystoma it has been impossible to obtain sufficient material to verify this point.

Whether the members of these Orthopteran unequal and other heteromorphic tetrads maintain their organization from one generation of animals to the next is yet to be demonstrated by breeding experiments now in progress in this laboratory. The expectation is that they do, since Wenrich ('16) and Carothers ('17) find every possible combination which would arise from the segregation and recombination of the members of these various types of tetrads.

The presence in the Orthoptera of unequal tetrads does not indicate a lack of individuality. On the contrary, the persistence of this condition throughout the individual, and perhaps from generation to generation, is strong evidence to the contrary. Of course a change has taken place at some time (if it be correct to assume that the homologues were all alike at some earlier period), but this is to be expected if these chromosomes are to parallel genetic behavior.

Bridges ('17, p. 445–6) presents parallel genetical data in connection with the chromosomes of Drosophila. He finds in certain cases that the genes for 'bar' eye and 'forked' bristles, whose loci are located near one end of the sex-chromosome, have been lost and that the region between these two loci has also been affected. He suggests that this deficiency may be due to a physical loss of this portion of the chromosome. He also reports ('19, p. 357) a case in which "a section of the X-chromosome, including the loci for vermilion and sable, became detached from its normal location in the middle of the X-chromosome and became joined on to the 'zero' end (spindle fiber) of its mate." In other instances the locus for sable alone, as

far as known, has been lost from one homologue and joined to the end of its mate. Another case is the transposition of a piece of the II chromosome to the middle of the III chromosome. He has exhibited definite cytological evidence (unpublished) supporting a part of the above. This condition produces homologues of unequal length which parallels the observations in the germ cells of the Orthoptera and the apparent similar condition in certain somatic homologues of Ambystoma.

c. Constant relative size relations. In addition to verifying Montgomery's ('01) and Sutton's ('02) observations of paired homologous chromosomes of equal length in the germ cells, Meek ('12), Robertson ('16), and Hance ('17 b, '18 a) confirm Sutton's ('02) observation (based upon comparisons of camera-lucida drawings of many spermatogonial cells and upon measurements of early prophase tetrads) that the proportional difference in size between any two pairs in one nucleus is practically the same as that between the corresponding pairs in any other nucleus. In Ambystoma tigrinum, as is seen in figures 33 to 37 and the table of percentages accompanying them, while the relative lengths are not exactly the same in every cell, there is in general a marked constancy of relative lengths. Were Meves' and Della Valle's measurements correct, the same would probably appear there.

d. Summary of measurements. The data here presented in connection with measurements upon the chromosomes of Ambystoma tigrinum and Salamandra maculosa cannot well be interpreted as a confirmation of Meves' and Della Valle's contention that pairing of the chromosomes and a constant organization of the chromosomes do not exist because: 1) Their data, on account of errors inherent in the material, are too unreliable to command confidence; 2) the differences in the lengths of the chromosomes of Salamandra maculosa are too small to permit one to deny or to affirm the existence of pairs, and, 3) it has been shown that because of obvious foreshortenings, individually mentioned above, which are unaccounted for in Meves' measurements, there probably is a somewhat constant difference between chromosomes 8 and 9 in eleven out of the twelve cells which he

measured. This confirms the contention of a constancy of chromatin organization so far as is possible in material having chromosomes differing so little in length as those of Salamandra maculosa. 4) The measurements of chromosomes in the somatic cells of Ambystoma tigrinum show duplication of sizes, especially pairs 1, 2, and 8 in figures 33 and 34, pairs 8, 9, and 10 in figure 36 and indications of the same in the other chromosomes of all the cells which differ too little in length to constitute reliable evidence. Explanations are offered for cases in which the homologues differ in length. 5) The chromosome lengths show approximately constant relative sizes in all of the cells measured.

Based upon the above considerations and upon the unequal and other heteromorphic tetrads in Orthoptera, my expectations are that the pairs and their relative lengths in the somatic cells of Ambystoma are constant for the individual, and although not exactly the same, they are approximately the same in different individuals. However, as stated above (p. 199), the measurements cannot be considered to demonstrate conclusively the presence of a duplicate series of chromosomes.

SUMMARY OF CONCLUSIONS

1. No variation is found in the somatic chromosome number of twenty-eight in Ambystoma tigrinum.

2. Della Valle's contention that variation in chromosome number is the rule is unconfirmed.

3. The chromosomes form approximately a duplicate series of sizes and forms, supporting the contention that they consist of pairs of maternal and paternal homologues.

4. An approximate constancy of size relations between pairs in the complexes of different individuals is also maintained.

5. Della Valle's claim that the chromosome lengths are a series of variants is not substantiated.

6. There is evidence of unequal homologues in these cells.

7. There is also a suggestion that there is a sex chromosome attached to a euchromosome.

8. There is apparently but little complete fragmentation of the chromosomes.

9. The above observations support the theory of the individuality of the chromosomes.

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1911 Studies on chromosomes. VII. A review of the chromosomes of Nezara, etc. Jour. Morph., vol. 22.

1912 Studies on chromosomes. VIII. Observations on the maturation phenomena in certain Hemiptera, etc. Jour. Exp. Zoöl., vol. 13.

WINIWARTER, H. VON 1900 Le corpuscle intermediare et le nombre des chromosomes chez le Lapins. Arch. de Biol., T. 16.

WOOLSEY, CARRIE I. 1915 Linkage of chromosomes correlated with reduction in numbers among the species of a genus, also within a species of Locustidae. Biol. Bull., vol. 28.

PLATES

EXPLANATION OF PLATES

The drawings were made with the aid of a camera lucida, using a Zeiss 2-mm. apochromatic immersion objective, N. A. 1.30, and a Spencer compensating ocular 20 × which produced a magnification of 2633. The illumination consisted of light from a 100-watt frosted-globe Mazda concentrated-filament lamp passed through a daylight glass or a common cobalt-blue glass filter, and an Abbe condenser. The observer was shaded from the light in front of him and from the sides by a black-cloth screen.

In reproduction plates 1 to 8 have been reduced one-third, and plate 9 three-fourths, giving a final magnification of 1755 and 1316, respectively.

Figures 1 to 20 represent complexes of class I; figures 21 and 23, complexes of class II.

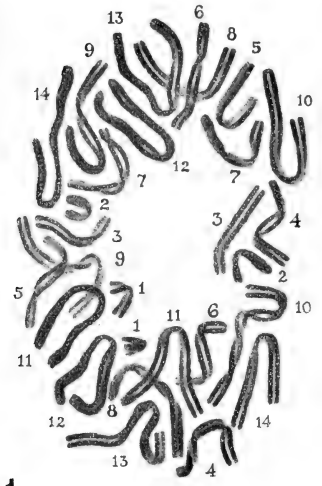
PLATE 1

EXPLANATION OF FIGURES

1 to 3 Very late peritoneal prophases.

4 A peritoneal metaphase.

The numbered pairs of chromosomes correspond to pairs bearing, respectively, the same numbers in figures 27 and 28, and in the legend of figures 33 and 34.


13
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12
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13
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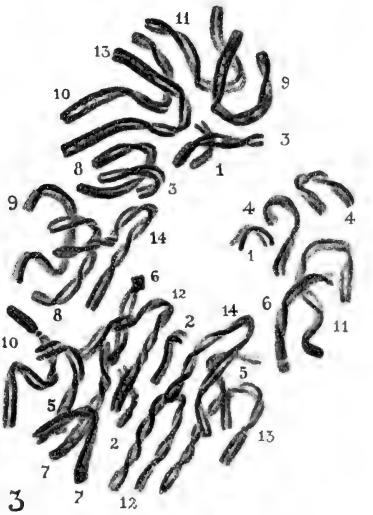
1

4

2

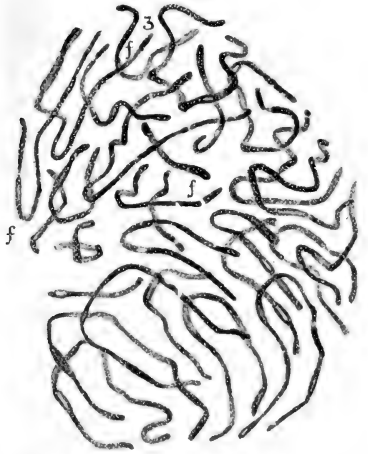

11
13
9
10
3
8
1
3
9
4
4
14
1
6
12
8
14
6
11
2
10
5
5
13
2
7
7
12

3

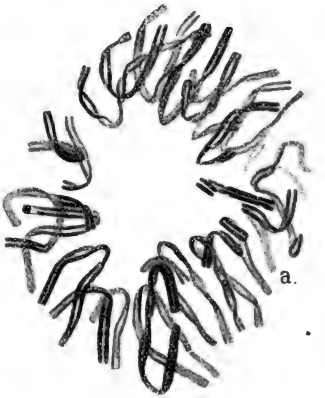
PLATE 2

EXPLANATION OF FIGURES

5 and 6 A gill-plate prophase and metaphase.
7 and 8 Metaphases of the tail epithelium.



5



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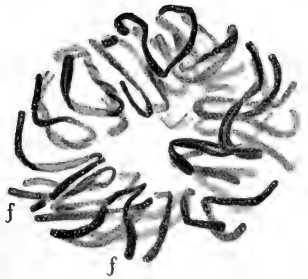
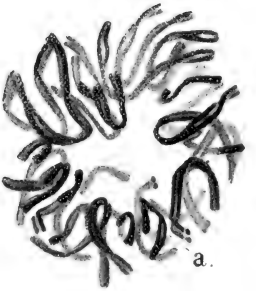


7



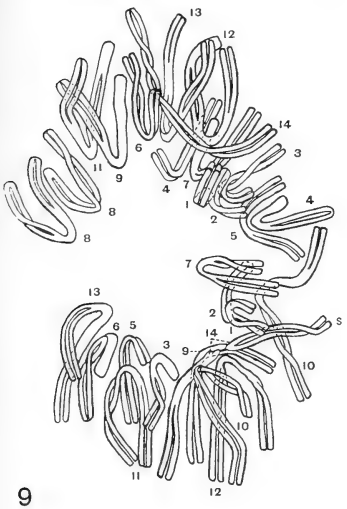
8

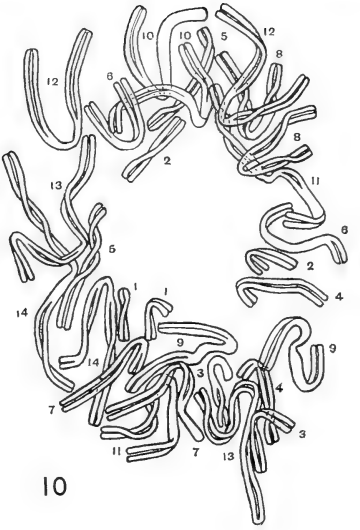
PLATE 3

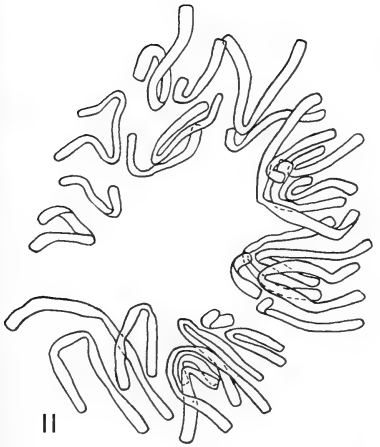
EXPLANATION OF FIGURES

9 to 12 Late prophase complexes of the lung epithelium.

The pair numbers correspond ,respectively, to those in figures 29 and 30, and in the legend of figures 35 and 36.


9


10


11

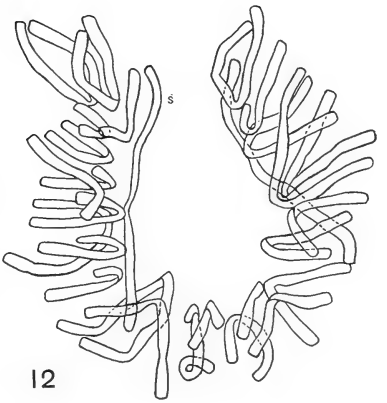
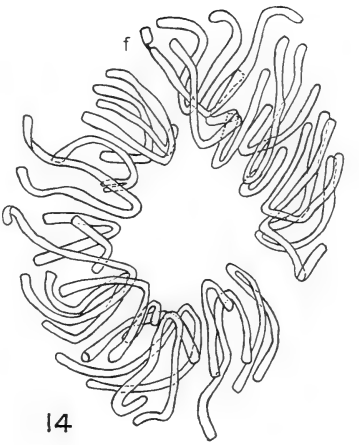

12

PLATE 4

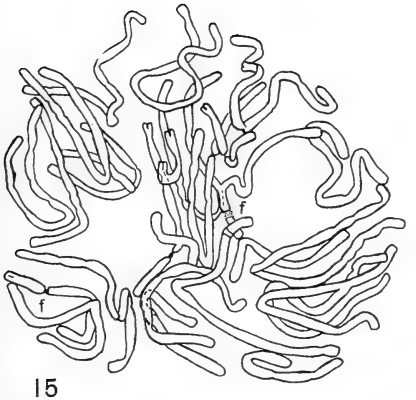
EXPLANATION OF FIGURES

13 to 16 Gill-plate complexes. The chromatids are shown only in figure 13.

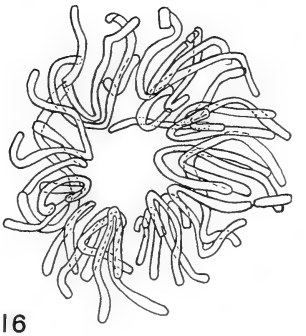
13



14



15

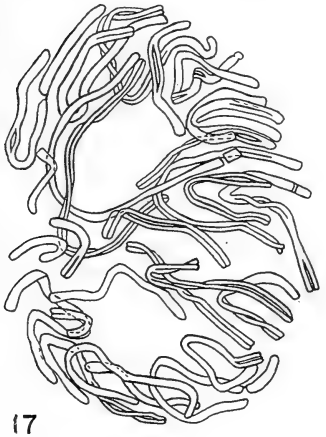


16

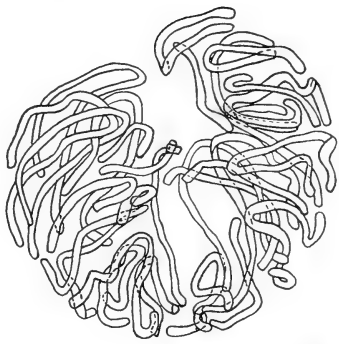
PLATE 5

EXPLANATION OF FIGURES

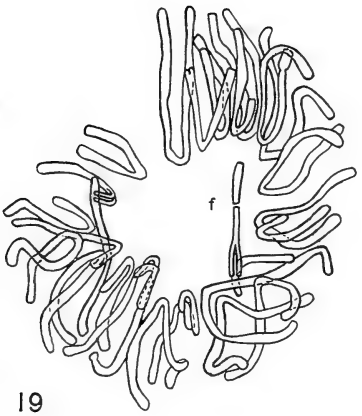
17 to 19 Two prophases and one early metaphase of gill-plate epithelium.
20 A peritoneal metaphase.



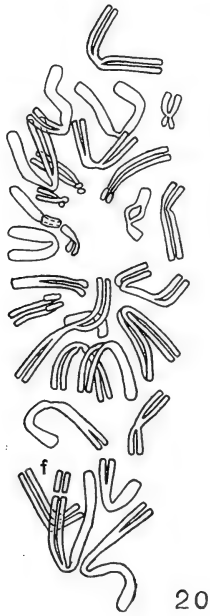
17



18



19



20

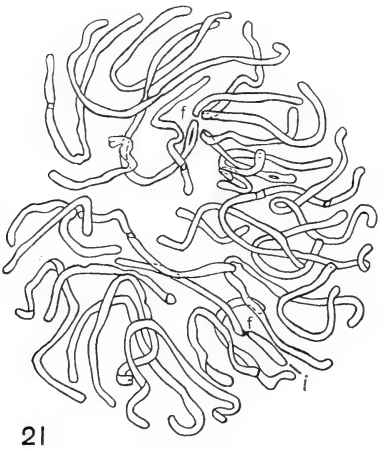
PLATE 6

EXPLANATION OF FIGURES

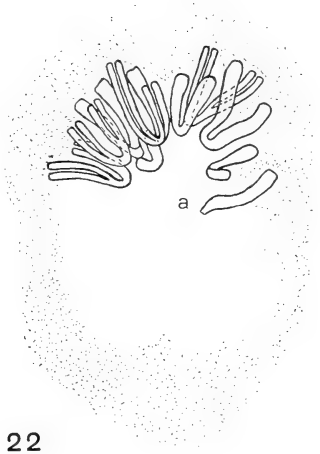
21 and 23 Gill-plate prophases of class II. Chromosomes '*f*,' figure 21, interpreted as one; '*i*,' figure 23, as two (p. 12).

22 A peritoneal complex with part of the chromosomes missing.

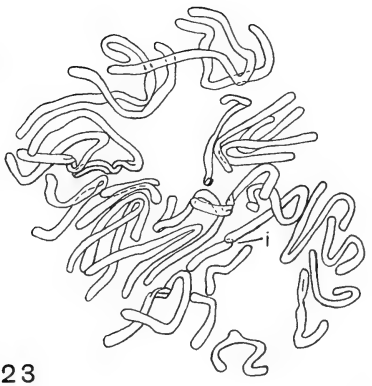
24 A peritoneal complex separated into two parts. The pair numbers are duplicated in figure 31 and in the legend of figure 37.



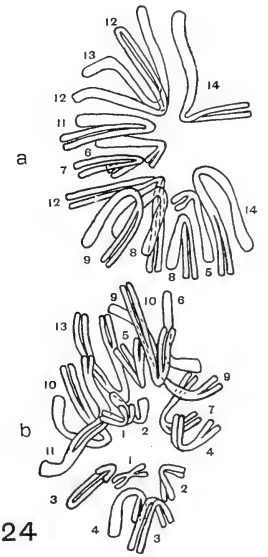
21



22



23



24

PLATE 7

EXPLANATION OF FIGURES

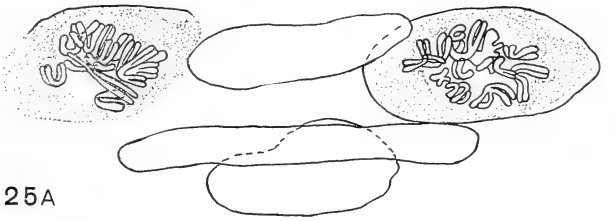
25A Another peritoneal complex separated into two parts which are drawn in their relative positions. × 866.

25B The chromosomes of figure 25A. × 1755.

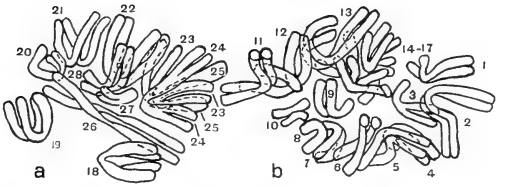
Chromosomes 14 to 17 are interpreted; note that the chromatids of these four chromosomes are well separated.

26A A lung epithelial cell separated into two parts which are drawn in their relative positions. × 866.

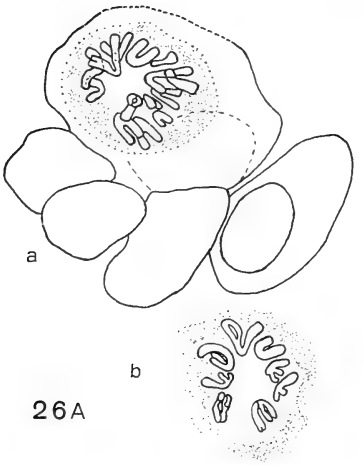
26B Chromosomes of 26A. × 1755. Pair numbers duplicated in figure 32.



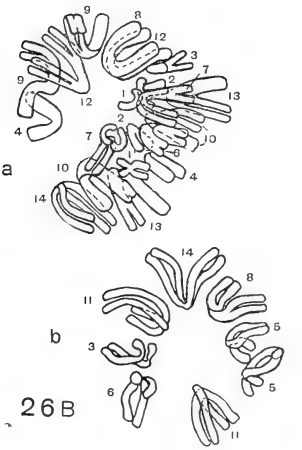
25A



25 B



26A



26B

PLATE 8

EXPLANATION OF FIGURES

27 to 32 Chromosomes of figures 1, 3, 9, 10, 24, and 26, respectively, arranged in pairs with pair numbers and figures indicating their lengths in millimeters at × 2633. The amount included in any figure for foreshortening is indicated above that figure. The pair numbers are duplicated in the above figures and in the legend of figures 33 to 37, respectively.

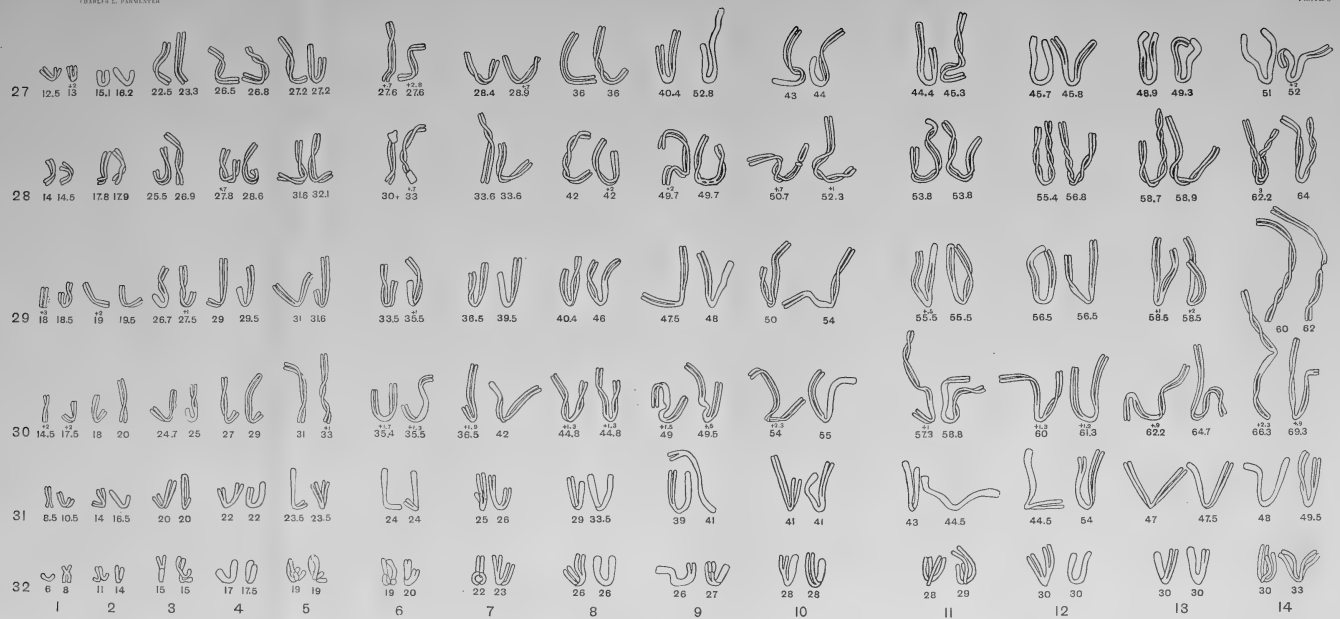

27
12.5 13
15.1 16.2
22.5 23.3
26.5 26.8
27.2 27.2
27.6 27.6
28.4 28.9
36 36
40.4 52.8
43 44
44.4 45.3
45.7 45.8
48.9 49.3
51 52
28
14 14.5
17.8 17.9
25.5 26.9
27.8 28.6
31.6 32.1
30, 33
33.6 33.6
42 42
49.7 49.7
50.7 52.3
53.8 53.8
55.4 56.8
58.7 58.9
62.2 64
29
18 18.5
19 19.5
26.7 27.5
29 29.5
31 31.6
33.5 35.5
36.5 39.5
40.4 46
47.5 48
50 54
55.5 55.5
56.5 56.5
58.5 58.5
60 62
30
14.5 17.5
18 20
24.7 25
27 29
31 33
35.4 35.5
36.5 42
44.8 44.8
49 49.5
54 55
57.3 58.8
60 61.3
62.2 64.7
66.3 69.3
31
8.5 10.5
14 16.5
20 20
22 22
23.5 23.5
24 24
25 26
29 33.5
39 41
41 41
43 44.5
44.5 54
47 47.5
48 49.5
32
6 8
11 14
15 15
17 17.5
19 19
19 20
22 23
26 26
26 27
28 28
28 29
30 30
30 30
30 33
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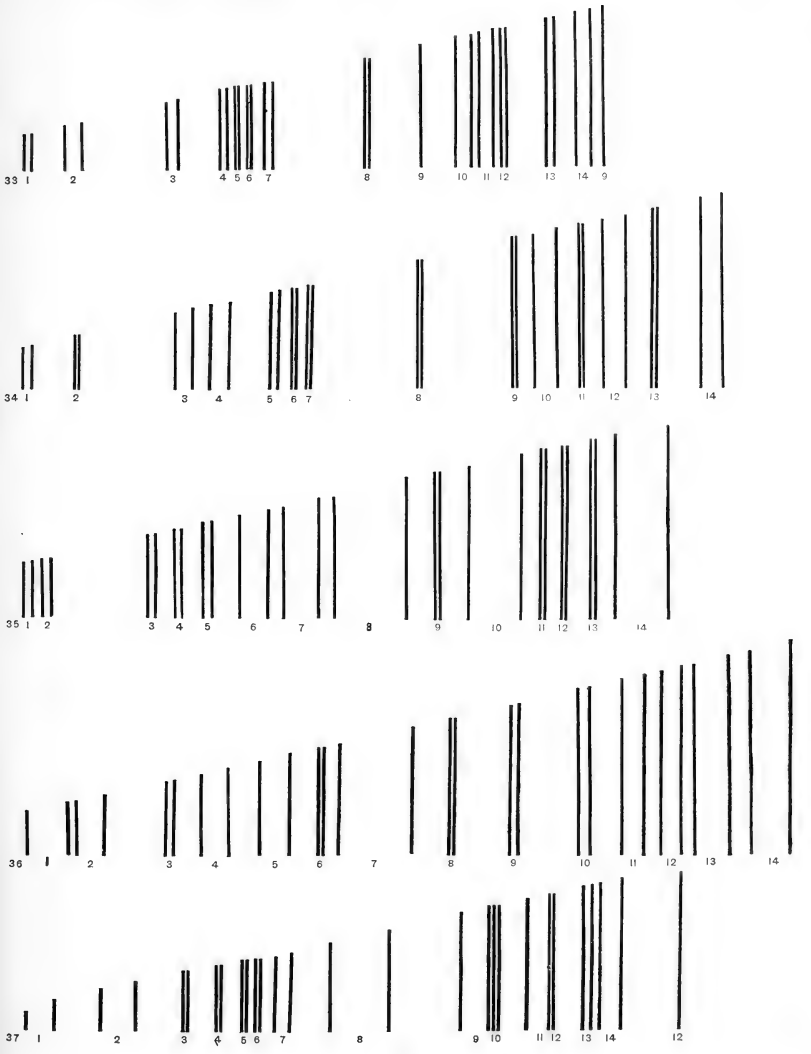
PLATE 9

EXPLANATION OF FIGURES

33 to 37 Representing the lengths of the chromosomes in the cells indicated in the table below at a magnification of 1316. The differences in the lengths of the lines and also the spaces between the lines represent relative differences in chromosome lengths. For convenience the width of the spaces between the lines are made eight times the differences in lengths. The lengths of the chromosomes and their percentage of the average length in the cell is shown in the table below. The amount of foreshortening in any chromosome is indicated in plate 8.

PAIRS	FIGURES									
	33 (1, 27)		34 (3, 28)		35 (9, 29)		36 (10, 30)		37 (24, 31)	
	Milli-metres	Per cent	Milli-metres	Per cent	Milli-metres	Per cent	Milli-metres	Per cent	Milli-metres	Per cent
1	12.5	37	14.0	35	18.0	44	14.5	34	8.5	27
	13.0	38	14.5	36	18.5	45	17.5	41	10.5	33
2	15.1	44	17.8	45	19.0	46	18.0	42	14.0	44
	16.2	47	17.9	45	19.5	47	20.0	47	16.5	52
3	22.5	66	25.5	64	26.7	65	24.7	58	20.0	63
	23.3	68	26.9	68	27.5	68	25.0	59	20.0	63
4	26.5	77	27.8	70	29.0	71	27.0	63	22.0	70
	26.8	78	28.6	72	29.5	72	29.0	68	22.0	70
5	27.2	80	31.6	79	31.0	76	31.0	73	23.5	75
	27.2	80	32.1	80	31.6	77	33.0	78	23.5	75
6	27.6	81	30+	—	33.5	82	35.4	84	24.0	76
	27.6	81	33.0	83	35.5	87	35.5	84	24.0	76
7	28.4	83	33.6	84	36.5	89	36.5	86	25.0	79
	28.9	84	33.6	84	39.5	97	42.0	99	26.0	81
8	36.0	105	42.0	105	40.4	99	44.8	103	29.0	91
	36.0	105	42.0	105	46.0	112	44.8	103	33.5	106
9	40.4	118	49.7	125	47.5	116	49.0	116	39.0	124
	52.8	154	49.7	125	48.0	117	49.5	117	41.0	130
10	43.0	126	50.7	127	50.0	122	54.0	127	41.0	130
	44.0	129	52.3	132	54.0	132	55.0	130	41.0	130
11	44.4	130	53.8	135	55.5	136	57.3	135	43.0	136
	45.3	132	53.8	135	55.5	136	58.8	138	44.5	141
12	45.7	134	55.4	139	56.5	138	60.0	142	44.5	141
	45.8	134	56.8	143	56.5	138	61.3	145	54.0	171
13	48.9	143	58.7	148	58.5	143	62.2	147	47.0	149
	49.3	144	58.9	148	58.5	143	64.7	153	47.5	150
14	51.0	149	62.2	156	60.0	147	66.3	156	48.0	152
	52.0	152	64.0	161	62.0	151	69.3	163	49.5	157


33 1 2 3 4 5 6 7 8 9 10 11 12 13 14 9
34 1 2 3 4 5 6 7 8 9 10 11 12 13 14
35 1 2 3 4 5 6 7 8 9 10 11 12 13 14
36 1 2 3 4 5 6 7 8 9 10 11 12 13 14
37 1 2 3 4 5 6 7 8 9 10 11 12 13 14 12

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JANUARY 19

THE ANATOMY OF THE HEAD AND MOUTH-PARTS OF ORTHOPTERA AND EUPLEXOPTERA[1]

HACHIRO YUASA

ONE HUNDRED AND SIXTY-THREE FIGURES (NINE PLATES)

CONTENTS

INTRODUCTION

Huxley ('78), with good reasons, turned to the generalized Orthoptera for a type for study and description as a representative of the class Hexapoda. Blatta orientalis was selected for this purpose, and its anatomy received a careful consideration. A few years later, Packard ('83) discussed rather briefly the homology of the head and mouth-parts of orthopterous and other insects. Miall and Denny ('86), three years later, published a book on the morphology and biology of the cockroach which is still considered a classic. The descriptive and theoretical aspects of the skeleton of the head of the more generalized insects, including the tentorium, were thoroughly studied by Comstock and Kochi ('02). After defining the sclerites and areas of the head-capsule, these writers attempted to ascribe each sclerite and appendage to some one of the seven primordial segments of which an insect's head was supposed to be composed. Riley ('04), who investigated the embryological development of the skeleton of the head of Blatta germanica, came to the conclusion that "so

[1] Contributions from the Entomological Laboratories of the University of Illinois, no. 55.

intimate a relation between the segmentation and the sclerites cannot be shown." More recently the mouth-parts of the cockroach were studied by Mangan ('08) and Bugnion ('13, '16).

In spite of the fact that an immense amount of valuable information is found in the more comprehensive works on general entomology, notably Kolbe ('89), Packard ('98), Henneguy ('04), Berlese ('06), and Schröder ('12), and, contrary to the general impression that the external anatomy of insects in general and especially of the more common insects such as Orthoptera has already received sufficient attention from morphologists, it is surprising, when the subject is scrutinized a little more closely, to find how few studies of homology have been attempted by the use of a series of forms. The possible exceptions in America are the successful attempts to homologize the wing veins of different orders by Comstock, Needham, MacGillivray, and others and the careful investigations of Crampton ('09, '14, '17), Snodgrass ('09, '10), and Peterson ('16) on the different regions of the insect body. The confusion in the terminology used by insect morphologists and the greater confusion of the anatomical terms now current among taxonomists, particularly among specialists interested exclusively in restricted groups, amply justify attempts to investigate and in many cases to reinvestigate the more fundamental structures of insects.

The following pages present a résumé of a detailed study of the salient characteristics of the external anatomy of the head and mouth-parts of the generalized biting insects as represented by typical species belonging to the orders Orthoptera and Euplexoptera. Particular attention was given to structures heretofore little studied—the prepharynx and tentorium. This study was undertaken under the supervision of Prof. Alex. D. MacGillivray, of the University of Illinois, and to him I extend my sincere thanks for suggestions and encouragement and for the permission to use his unpublished terminology[2] and forthcoming outline for the study of insect anatomy. This outline proved most valuable and indispensable as a guide.

[2] The new terms used in this paper are defined in the following pages. On pages 285–286 they are tabulated in their relations to other parts.

MATERIAL AND METHODS

In order to make the study as comprehensive and representative as possible, the following species have been selected and examined: Blatta orientalis (Blattidae), Mantis religiosa (Mantidae), Diapheromera femorata (Phasmidae), Gryllus pennsylvanicus (Gryllidae), Orchelimum vulgare (Locustidae), Stenopelmatus sp. (Locustidae), Melanoplus differentialis (Acrididae), Tettix arenosus (Acrididae), and Anisolabis maritima (Euplexoptera).

The specimens were treated, as a rule, with a 5 per cent solution of potassium hydroxid from five to twenty-four or more hours (depending upon the degree of chitinization of the structures) and examined in 70 per cent alcohol under a binocular microscope. The drawings were made free hand after measuring the dimensions of the specimens by means of an ocular micrometer.

OBSERVATIONS

A. Fixed parts of the head

The conditions in the cockroach have been taken as typical, and those of other families have been discussed only where they differ from this type. Detailed descriptions have been omitted; with the accompanying figures it is believed that no difficulty will be experienced in identifying on specimens the structures shown. In order to avoid confusion, the skeleton of the head and its appendages have been described in every case as if the mouth were directed cephalad.

The epicranial suture in Blatta (fig. 1) is the inverted Y-shaped median suture. The stem (*es*) of the Y begins at the occipital foramen, extends cephalad on to the dorsal aspect for some distance, then bifurcates. Each arm (*ea*) extends obliquely toward an antacoria and terminates in the whitish area mesocaudad of the latter. The epicranial suture is well developed in Gryllus (fig. 5) and Anisolabis (fig. 12); in the former, however, the caudal portion of the stem is obsolete in untreated specimens, and the arms are short and terminate at the mesal margins of the

lateral ocelli, while in the latter, the epicranial arms are very conspicuous, semicircular, and extend to the middle of the mesal margins of the compound eyes and apparently continue cephalad to the lateral margins of the antacoriae. In Mantis (figs. 3, 4), Stenopelmatus (fig. 6), and Melanoplus (fig. 10), the stem is distinct, represented by a parademe in the latter. The stem is represented in Orchelimum (fig. 7) by a longitudinal median furrow which becomes obsolete near the dorsal margin of the caudodorsal median prominence of the epicranium. In Diapheromera (fig. 8) the stem is the long faint line, seen only in treated specimens, which bifurcates between the antacoriae. It is obsolete in Tettix (fig. 11) and its position is indicated by a short parademe on the caudal aspect of the head. In Mantis the epicranial arms are represented by the parademe caudad of the lateral ocelli; they are very short and faint in Diapheromera; obsolete in Orchelimum, although the deep transverse furrow at the cephalic margin of the epicranial prominence may represent them. In Stenopelmatus their position is indicated by the parademes which terminate near the mesal margins of the antacoriae. They are short in Melanoplus and bend caudad between the caudal ends of the compound eyes; obsolete in Tettix, their position is indicated by a short curved parademe between the compound eyes.

The frontogenal suture (*fgs*) in Blatta extends laterad for some distance from the lateral end of each pretentorina, then caudad toward the cephalomesal angle of the compound eye, where it merges into a short furrow which originates near the caudolateral margin of the antacoria. It is only slightly curved in Mantis and Gryllus, becoming obsolete near the cephalic part of the mesal margin of the compound eye; it is very short in Diapheromera and terminates near the middle of the cephalic margin of the eye; is depressed in Melanoplus and short in Tettix and terminates at the cephalomesal angle of the eye; in Anisolabis apparently merges into the cephalic end of each epicranial arm near the antacoria, and is wanting in Orchelimum and Stenopelmatus. The frontogenal sutures are considered as the cephalic portions of the epicranial arms which have been isolated

from the caudal portions by the encroachment of the antacoriae and lateral ocelli.

The vertex (*v*) in Blatta (figs. 1 and 13) extends on the caudal and dorsal aspects from near the occipital foramen to the epicranial arms, and is divided on the meson by the epicranial stem. On the lateral aspect each half of the vertex extends cephalad between the compound eye and the occipital suture to the mandibularia (*mb*), then dorsad to the frontogenal suture, and is connected with the dorsocaudal part by a narrow area between the compound eye and the antacoria. The vertex, therefore, occupies nearly one-third of the surface of the head and includes the compound eyes and lateral ocelli.

The extent of the vertex depends upon the shape and size of the head and the position of the epicranial suture. It reaches its maximum development in Diapheromera and is much restricted on the caudal aspect in Orchelimum and Tettix. The lateral ocelli are not situated on the vertex in Mantis, Melanoplus, and Tettix. A furrow with an accompanying parademe extends cephalodorsad from the occipital foramen on each lateral half of the vertex in Mantis, Diapheromera, and Anisolabis. Whether these furrows are homologous with the vertical furrows found in the larvae of the Tenthredinidae and other Entometabola is not known.

The portion of the vertex cephalad of each compound eye is a gena (*g*). It is restricted in Mantis and Diapheromera, ample in Melanoplus and Tettix, and fused with the front in Orchelimum and Stenopelmatus. In Anisolabis it is completely isolated from the rest of the vertex by the cephalic encroachment of the occipital suture.

The compound eyes (*ce*) in Blatta are large, kidney-shaped, emarginate on the mesal margin, and occupy the dorsal, lateral, and caudal parts of the head. They are present in all the genera studied, but vary in size, shape, and position.

The narrow annular sclerite surrounding the periphery of each compound eye is the oculata (*ol*). It is produced entad as a ring-like plate (fig. 45). Oculatae are always present and the ental rings are more or less well developed, reaching the maxi-

mum size in the species having large compound eyes, as Mantis (fig. 46) and Melanoplus (fig. 49).

The lateral ocellus (*lo*) in Blatta (fig. 1) is in the smooth oblong area located in the whitish spot in which each epicranial arm terminates; the median ocellus (*mo*) is wanting. In other species the ocelli vary in size, shape, number, and position (figs. 4, 5, and 10). In Diapheromera the median ocellus is wanting, while in Stenopelmatus and Anisolabis all ocelli are absent. When the usual number of the ocelli, three, is present, they are arranged in a triangle as in Mantis. There is a sexual dimorphism in this genus (figs. 3 and 4).

The front (*f*) in Blatta is well developed and is bounded by the epicranial arms, antacoriae, and frontogenal sutures on the caudal and lateral aspects, respectively. The cephalic boundary is an imaginary line connecting the mesal ends of the frontogenal sutures or the pretentorinae. A smooth oblong spot, mesocephalad of each antacoria, is a muscle impression (*mi*). The front varies in extent and position. Its cephalic boundary is marked by the frontoclypeal suture (*fcs*) in all, except Orchelimum and Stenopelmatus where the lateral boundaries are also indefinite. It includes all or part of the antacoriae, frequently the lateral ocelli, and the median ocellus when present. Muscle impressions on the front, which have been mistaken for ocelli, occur in Gryllus, Melanoplus, Tettix, and Anisolabis (fig. 12). Tettix has a prominent inverted Y-shaped ridge on the front, which is not connected with the frontoclypeal suture.

Frontoclypeal sutures (*fcs*) are present except in Orchelimum and Stenopelmatus.

The antacoriae (*an*) are the circular membranous areas in which the antennae are inserted. They are adjacent to and mesad of the compound eyes in all the genera studied, except Anisolabis where they are cephalad of them. They are located on the front, except in Diapheromera and Stenopelmatus where the position of the epicranial arms would indicate that they belong to the vertex.

The antennaria (*ar*) in Blatta is the chitinized annular sclerite forming the periphery of each antacoria. Its inner margin is

connected with the flexible membrane which is attached to the scape of the antenna. There is a narrow pointed projection, antacoila (*aa*), extending caudad from the cephalic margin of the antennaria (fig. 54). This is the 'chitinized pin' of Miall and Denny ('89). Another fine slender bar is attached to the caudal margin of the scape. These two projections, together with the antatendons (*at*) control the movements of the antenna. The minute chitinized spots (*ch*) in the antacoria mark the attachment of these tendons. The antennariae are always present. The articulation of the antennae is either like that of the cockroach, as in Mantis (fig. 56) and Anisolabis (fig. 61); by means of less definite projections, as in Gryllus (fig. 60), Stenopelmatus and Melanoplus, or by means of the triangular projection of the scape which fits into the emargination of the antennaria (fig. 62). There are two antatendons, and their points of attachement can be identified in all the genera.

The pretentorina (*pn*) in Blatta (figs. 1 and 2) is the linear, transverse furrow along the caudomesal margin of each mandibularia. In all the genera studied, with a possible exception of Anisolabis, the pretentorinae are distinct and definite in their location and afford excellent dependable landmarks in orienting other structures. Although they are always associated with the front, mandibularia, frontogenal and mandogenal sutures, they differ somewhat in their extent and appearance. The lateral or caudal portion of each pretentorina terminates either on the frontogenal suture, more or less remote from the mandibularia, as in Blatta, Mantis, and Anisolabis, or on the mandogenal suture beyond the cephalic end of the frontogenal suture, as in Gryllus, Melanoplus, and others.

The suture forming the caudal margin of each mandibularia and separating it from the vertex or gena and front is the mandogenal suture (*mgs*). This suture is present, except in Anisolabis, but varies in length and direction on account of the differences in size, shape, and position of the mandibularia.

The mandibularia (*mb*) in Blatta (fig. 2) is the small triangular area extending from the precoila to the cephalic end of the occipital suture and, on its cephalic margin, which is often submem-

branous, is connected with the proximal portion of each mandible. The extensacuta (*ec*) is located near the ventral part of the cephalic margin of the mandibularia. The mandibulariae, although they vary in size and shape, are present and more or less well differentiated in all except in Anisolabis (fig. 17).

The occipital foramen (fig. 23, *of*) is the large subquadrate opening located in the caudal part of the ventral aspect of the head. It varies in size, shape, and position. The elongation and the peculiar position of the foramen in Diapheromera (fig. 29) are due to the natural position of the head which is horizontal instead of vertical as in the case of the other genera.

In Blatta (fig. 23) the occipital suture (*os*) is distinct; it begins at the lateral end of each postcoila and extends caudad, becoming obsolete some distance from and slightly beyond the caudal margin of the occipital foramen. The occipital sutures are present in all except Diapheromera. They are practically complete in Gryllus (fig. 28) and Tettix (fig. 35) where the caudal ends of the sutures unite with the epicranial stem near its origin. In other genera the caudal ends of the sutures are either free, as in Melanoplus (fig. 30), Orchelimum (fig. 27), and Stenopelmatus (fig. 31), or they merge into the furrows which extend on to the vertex, as in Mantis (fig. 24) and Anisolabis (fig. 32). In Mantis (fig. 41), Orchelimum (fig. 44), and Melanoplus (fig. 49) the sutures are connected with the lateral margins of the occipital foramen by an ental thickening or parademe (*pm*). The cephalic portion of each occipital suture is produced as a parademe in Gryllus and others.

The occiput (*oc*) in Blatta (fig. 23) is the narrow crescentic area surrounding the caudal one-third of the occipital foramen. Since there is no suture (excepting the occipital suture on the lateral boundaries) either on its caudal margin where it meets the vertex or on its cephalic margin where it merges with the postgenae, the exact boundaries of the occiput cannot be established. The imaginary line drawn across the occipital foramen just cephalad of the odontoidea (*od*) is here considered as the cephalic limit of the occiput. In other genera the occiput varies in size and shape and its caudal and cephalic boundaries are

more or less indefinite. In Melanoplus and others the oblique parademes mentioned above form its cephalic margin.

The postgenae (*pgn*) in Blatta (fig. 23) is the flat area cephalad of the occiput and mesad of each occipital suture. The caudal half of its mesal margin is bounded by the lateral margin of the occipital foramen and the cephalic half is roundly emarginate and continuous with the microcoria (*ma*). The postcoila (*ptl*) is located on the cephalic margin. The relation of each postgena to the adjacent areas is similar in all the genera studied, but it varies in size and shape due largely to the difference in size and shape of the occipital foramen. There is a distinct crassa (*cr*) adjacent to and parallel with the cephalic part of the mesal margin of each postgena which extends from near the middle of the postcoila in Gryllus (fig. 28) and from the lateral end in Anisolabis (fig. 32). On the ental surface of the crassa, there is a corresponding parademe. This parademe is present in all and extends from each postcoila to the occipital foramen along the mesal margin of each postgena.

The postcoila (fig. 23, *ptl*) is the blackish acetabulum in which the postartis of the mandible articulates. It is located at the cephalic margin of each postgena. It is usually distinct and well developed, being largest in Gryllus, comparatively shallow in Diapheromera, and located on the mesal angle of the cephalic margin of the postgena in Melanoplus and Tettix.

The metatentorina (*mn*) in Blatta (figs. 13 and 23) is the elongated opening located on each side of the cephalic margin of the occipital foramen between the maxillaria and the postgena and leading into the corpotentorium. It is always present and distinct and similar in position, but varies in size and shape.

The paracoila (*pl*) in Blatta is the condyle-like projection formed by the cephalolateral angle of the maxillaria protruding ventrocephalad on each side of the occipital foramen. It is folded longitudinally upon itself, the crest of the fold forming a condyle and the concavity an acetabulum. The margin, near the lateral portion of each paracoila, is emarginate and forms the other side of the acetabulum. The exparartis of the maxilla is articulated against this condyle and acetabulum. The para-

coilae are always present and distinct. They are not well differentiated in Diapheromera, but are prominent mesocaudal projections in Tettix.

The maxillariae (*my*) in Blatta (fig. 23) are narrow plates surrounding the lateral and caudal margins of the occipital foramen. The ectal surface of each maxillaria is closely applied to the ectal surface of the postgena and the occiput. The lateral margins are folded, forming a roll. The caudal part of each roll is produced into a cone-shaped projection, an odontoidea (*od*). The microcoria (*ma*) is attached to the ventral margin of the maxillariae. The caudal side of each maxillaria is reduced to a narrow band, the cephalic part of each lateral portion is expanded and produced to form a distinct paracoila, and the cephalomesal margin is fused with the corpotentorium. The maxillariae are simple in Orchelimum, Melanoplus, and Tettix. In other genera they vary in size and shape, are very complicated and have, besides odontoideae, many projections, some of which bear tendons (figs. 27 and 31).

The odontoideae (*od*) are not well differentiated in Orchelimum and Melanoplus, but are distinct in the other genera. In Blatta, Stenopelmatus, and Anisolabis they occur on the caudal third of the lateral parts of the maxillariae; in other genera near the cephalic part of the maxillariae and extend ventrad or caudoventrad of the corpotentorium.

The clypeus (*c*) in Blatta (fig. 1) is the convex sclerite attached to the cephalic margin of the front. The caudolateral angles are produced into thickened, transverse lobes, and each bears a distinct precoila. The lateral margins are rounded and the cephalic boundary is the distinct clypeolabral suture. The clypeus is transverse, the lateral margins are entire and converge cephalad. In Gryllus (fig. 5) and Melanoplus (fig. 10) there are oblique furrows extending from the middle of the lateral margins, which may represent the incomplete clypeal suture (*cs*) dividing the clypeus into preclypeus and postclypeus. In Diapheromera (fig. 8) the mesal part of the clypeus is dilated and produced, forming a prominent elevation.

The clypeolabral suture (*cls*) is distinct and complete in all, except Orchelimum, where it is represented by a transverse fold; in Stenopelmatus the mesal portion and in Diapheromera the lateral portions are obsolete. The suture in Melanoplus is irregular, the clypeus is emarginate on the meson.

The laterocaudal angles of the clypeus are produced into small lobes which are emarginate on the cephalic and ventral aspects. These emarginations form the precoilae in which the preartes of the mandibles articulate. The precoilae (*pr*) are distinct and practically similar in all. They are conspicuous in Mantis, Diapheromera, and Stenopelmatus (fig. 6).

The small chitinized structures at the lateral ends of the clypeolabral suture are the dorsal parts of the tormae (*tm*). They extend on to the ventral aspect and are the landmarks indicating the boundary between the clypeus and labrum. Tormae are present in all the genera studied.

The labrum (*l*) in Blatta is flexible, its lateral margins are rounded, and its cephalic margin is emarginate. The emargination is marked on each side by an oblique blackish thickening and bears spinulae. The labrum, although varying in size and shape, is distinct and well developed in all.

The tentorium is the endoskeleton of the head and is always well developed. Its form is closely related with and to a large extent influenced by the development and direction of the mouthparts. It is expanded and comparatively thin in Blatta (fig. 36); thick and heavily chitinized in Mantis (fig. 41), Gryllus (fig. 37), Stenopelmatus (fig. 43), Melanoplus (fig. 39), and Anisolabis (figs. 32 and 42). The cephalic portion is reduced in Diapheromera (fig. 38) and elongated and enlarged in Anisolabis. The location of the external markings or invaginations of the arms of the tentorium have been described elsewhere. The tentorium in Blatta (figs. 36 and 45) is composed of the typical parts, namely, the metatentoria (*mt*), corpotentorium (*ct*), pretentoria (*pt*), laminatentorium (*lt*), and supratentoria (*st*). The corpotentorium is the cuticular plate connecting the postgenae within the head; it is formed by the fusion of the mesal portions of the metatentoria. The ental plate surrounding the lateral and caudal

margins of the occipital foramen are considered as belonging to the metatentoria. The fan-shaped plate connected with each pretentorina is a pretentorium. Its cephalic margin is thickened, and its dorsomesal margin is thickened and turned mesad. The expanded caudal parts of the pretentoria converge, fuse on the meson, separate bounding a small opening, and then fuse with each other and the cephalic portion of the corpotentorium. The quadrate plate, the cephalic part of which is arched, is the laminatentorium. The two tendinous projections which extend cephalad from the opening in the tentorium are the oesotendons (*ot*). The circular opening is the foramen through which the nerve cord passes. The line of fusion of the pretentoria on the meson is indicated by a ventral ridge located cephalad of the opening. The thin, cuticular, triangular plate continuous with each caudolateral margin of the laminatentorium is a supratentorium. The apex of the triangle is produced into a thin, delicate, slender extension which is directed laterodorsad toward an antacoria. The point of attachment could not be determined, but it has been stated that it is attached to the caudolateral margin of the antennaria.

The metatentoria are always distinct and afford excellent landmarks for beginning the study of the tentorium. The corpotentorium connects the postgenae and forms the cephalic margin of the occipital foramen. It is usually vertical in position, but in Anisolabis it is a narrow horizontal plate. Each metatentorium fuses with a postgena on the dorsal side and with a maxillaria on the ventral, and extends as a more or less flaring band along the lateral and caudal margins of the occipital foramen. The flaring inner margins are produced as tendons (figs. 23, 28, and 32).

The pretentorium is generally fan-shaped or expanded where it is attached to the pretentorina on the ental surface of the frontogenal suture. Each is directed caudomesad for a short distance, then is twisted, again expanded, extends to the meson, and fuses with the other pretentorium, and forms a more or less distinct laminatentorium. The pretentorium in Mantis is turned caudad and follows the frontogenal suture to its caudal end. The

fan-shaped portion is divided into a large, concave mesal part and a small, triangular, cephalolateral part. A somewhat similar condition occurs in Gryllus, where the median carina is small and affects the contour of the pretentorium only slightly. In Diapheromera and Orchelimum a lateral furrow extends longitudinally and tends to fold dorsad forming the lateroventral margin. In Stenopelmatus, Orchelimum, Melanoplus (fig. 39), Tettix (fig. 40), and Anisolabis (fig. 42) the dorsomesal margin is thickened and the lateral expansion concave. This concavity reaches its maximum development in Anisolabis, where it forms a cup-shaped pocket along the frontogenal suture and the antennaria.

The laminatentorium is usually well developed and its caudal margin is often constricted and fused with the corpotentorium. It is small in Diapheromera and almost wanting in Orchelimum and Stenopelmatus. The line of fusion is distinctly indicated in Mantis, Diapheromera, Gryllus, and Tettix. In Anisolabis the line of fusion extends to the caudal margin of the corpotentorium. There is a circular opening in the laminatentorium of Mantis, as in Blatta, but the oesotendons are wanting. The laminatentorium is triangular and concave in Gryllus and Tettix and emarginate on the dorsal aspect in Melanoplus. The plate-like projection on each mesal side of the pretentorium in Orchelimum (fig. 43) and Stenopelmatus (fig. 44) may represent the vestige of the laminatentorium. The dorsomesal thickenings of the pretentoria are frequently continuous with the dorsolateral thickenings of the metatentoria, forming the X-shaped dorsal ridges across the laminatentorium and corpotentorium. In Anisolabis, however, this connection is obsolete and the caudal part of the laminatentorium is elongated, an inverted trough-shaped structure, with a longitudinal depression on each side instead of a dorsal thickening.

The supratentoria arise from the cephalic part of the dorsolateral margins of the laminatentorium in all, except in Diapheromera (fig. 51) where they issue from the pretentoria. They are always small, linear, and expanded, adjacent to the laminatentorium; and in Gryllus (fig. 47) they are attached to the caudo-

lateral angles of the antennariae; in Tettix (fig. 52) they are attached on the front cephalolaterad of the antacoriae; and in Anisolabis (fig. 53) near the caudomesal margins of the compound eyes. In others they are directed laterodorsad or laterodorso-caudad toward the antacoriae or points near them and are apparently unattached.

B. Movable parts of the head

The antennae (*a*) of Blatta (figs. 54 and 55) are long, slender, setaceous, and multisegmented. The scape (*sc*), pedicel (*p*), and the segments of the flagellum (*fl*) are cylindrical and setigerous. The proximal margin of the scape is emarginate, forming an antartis (*ad*), and articulates with the antacoila (*aa*). Two antatendons (*at*) are attached to the scape near the emargination. The first segment of the flagellum is longer than the pedicel in the female and shorter in the male. The antennae of other species are either filiform or setaceous and articulate with the antennaria. The scape is quadrate and flattened in Gryllus (figs. 59 and 60) and elongated in Anisolabis (fig. 61).

The mandibles (*md*) in Blatta (figs. 70, 72, and 73) are convex on the dorsal aspect and concave on the ventral. The preartis (*py*) is a combination of condyle and acetabulum, and is located on the dorsal aspect, while the postartis (*ptc*) is globular, prominent, and situated near the lateral margin of the ventral aspect. The small spatulate extensotendon (*et*) is attached near the postartis and the large branched rectotendon (*rt*) to the proximal part of the mesal margin on the ventral aspect. The acia (*ac*) is well differentiated and the mola (*ml*) is the triangular tooth cephalad of the acia. The dentes (*d*) are sharp and three in number in the dextral mandible and five in the sinistral. The mandibles are always strongly chitinized and fitted for cutting and grinding. They are so constructed that they interlock when at rest. Asymmetry of various degrees exists between the dentes and concavities. Aside from such differences, the mandibles are similar in form on both sides and more or less similar in both sexes. They usually differ, in different species, in size, shape, and manner of interlocking, but most of the structures in the

cockroach can be identified. The acia is generally absent, although the digit-like cuticular projection (*ac*) in Mantis (figs. 74, 75, and 76) may represent it. The base of the rectotendon is more or less distinctly chitinized and forms, in many cases, a sclerite-like area, the rectacuta (*rc*) of the ambipharynx. The dentes are always present, but are not differentiable into proxadentes (*pd*) and distadentes (*dd*) except in Anisolabis (figs. 86 and 87). The dentes and mola cannot be distinguished in Diapheromera (figs. 80 and 81).

The maxillae (*mx*) in Blatta (figs. 97 and 100) are typically orthopterous in structure and each consists of a cardo, stipes, galea, lacinea, palpifer, and maxillary palpus. The cardo (*ca*) consists of two segments (fig. 98), the subcardo (*sa*) and alacardo (*al*). The parartis is dorsal in position and bifurcate, the exparartis (*ey*) is a combination of a condyle and acetabulum. The entoparartis (*en*) is triangular, prominent, and bears the small premaxatendon (*pmt*) near its distal end. The alacardo is much smaller than the subcardo and attached obliquely to the lateroventral margin of the latter. The suture between these segments is distinct on the ventral aspect and forms a parademe. The exparartis articulates with the paracoila and the entoparartis is inserted near the paracoila dorso entad of the mesal margin of the head. The stipes (*s*) is large, distinctly chitinized on the ventral aspect, and, excepting a triangular area, is largely membranous on the dorsal aspect. It articulates with the alacardo at the caudolateral angle of the latter and is separated from it by a distinct suture. The caudomesal angle of the stipes on the ventral aspect articulates with the laterocephalic angle of the subcardo. Thus the stipes has two points of articulation with the cardo, one on the subcardo and the other on the alacardo. The narrow longitudinal sclerite attached to the mesal margin of the stipes on the ventral aspect is the subgalea (*sg*). The suture between them is modified into a distinct parademe. On the ventral aspect the cephalic margin of the subcardo and the mesal margins of subgalea, and of the stipes, cephalad of the subgalea, are continuous with the labacoria (*lc*), while the cephalic and mesal margins of the cardo and stipes, respectively, are continuous on

the dorsal aspect with the maxacoria (*mc*). The palpifer (*pf*) is the small sclerite on the dorsal aspect, near the cephalolateral part of the stipes and is separated from it by a subchitinized area. The galea (*gl*) is two-segmented. It is attached to the laterodistal margin of the stipes without any indication of a suture. The proxagalea (*pg*) is short, subcylindrical, and its distal margin on the dorsal aspect is marked by a transverse chitinous band and on the ventral by a distinct fold. The distagalea (*dg*) is much longer and slightly narrower than the proxagalea. It is hood-shaped and overlaps the distal portion of the lacinia. The distomesal margin is flaring and spinulate. The lacinia (*la*) is the flattened, chitinous, distal appendage mesad of the galea which fits into the mesal concavity of the latter. The lacinia is separated from the stipes by the distinct suture already mentioned, but its caudolateral portion is expanded into a subquadrate area which encroaches upon the stipes, and the suture is obliterated for a short distance. The suture is obsolete on the dorsal aspect. The maxadentes (*ms*) are sharp, curved, strongly chitinized, and two in number. The hamadens (*h*) is located near the maxadentes and is minutely tridentate. The mesal margin of the lacinia is convex, sharply flattened, and bears prominent lacinarastra (*rs*). The maxillary palpus (*mp*) is five-segmented and the mesocephalic margin of the distal segment is membranous and covered with setae and spinules.

The cardo is two-segmented in all species studied. The subcardo is irregular in outline, its entoparartis is always prominent and bears the premaxatendon, and its exparartis is often bifurcate and combines the function of a condyle and an acetabulum. The alacardo is convex, triangular or semitriangular, and obliquely attached to the subcardo. The ental surface of the suture between the two segments bears a parademe. The alacardo articulates with the stipes at its laterodistal angle, and the subcardo with the caudomesal angle of the stipes on the ventral aspect. In Mantis (figs. 96 and 104) and Anisolabis (figs. 93, 94, and 95), the alacardo is triangular, smaller than the subcardo. In Mantis, Diapheromera (figs. 110, 115, and

116), Stenopelmatus (figs. 101, 109, and 111), and Anisolabis the subcardo extends along the caudal and dorsal margins of the alacardo. In Gryllus (figs. 99, 102, and 103), Orchelimum (figs. 105, 107, and 108), Melanoplus (figs. 112, 113, and 114), and Tettix (figs. 117, 118, and 119) the subcardo is deeply emarginate and surrounds the alacardo on the ventral, caudal, and dorsal aspects. In these genera the suture between the two segments is obliterated, probably due to the irregularity produced by the complicated parademes. In Diapheromera and Melanoplus the exparartis is entire and in other genera it is shallowly or broadly bifurcate.

The stipes, except in Anisolabis, is subquadrate and chitinized on the ventral aspect; on the dorsal aspect the chitinous area is small and triangular, and the remainder of the surface is membranous or submembranous. In Anisolabis the stipes is divided into two parts, the proximal of which is small and triangular and located on the ventrolateral aspect, the distal is triangular and located on the ventral aspect. They are separated on the ventral aspect by the encroachment of the greatly enlarged subgalea (fig. 94, *sg*) and on the dorsal aspect by the elongated palpifer (fig. 93, *pf*); the two parts are connected by a narrow membranous area.

The subgalea is always present, is narrow, and extends longitudinally, except in Anisolabis where it is very large, flat, subpentagonal, and occupies the greater part of the ventral surface. It extends along the entire mesal margin of the stipes in Mantis, Gryllus, Stenopelmatus, and Anisolabis. In the others it does not quite reach the cephalic margin of the stipes. The suture between the stipes and subgalea is distinct except in Mantis, where a furrow marks the line of fusion of the two sclerites and its ental surface is thickened.

The palpifer is uniformly present and in Mantis and Diapheromera it is very indistinctly separated from the stipes. It is distinct, although on the dorsal aspect it is submembranous and its boundary is more or less obliterated, except in Anisolabis, as already noted. In Melanoplus it is confined to the ventral aspect.

The lacinia is hook-shaped, depressed, chitinized, and dentate in all; its surfaces and the mesal margin are convex. In Mantis, Gryllus, Orchelimum, and Stenopelmatus, the lateral half of the proximal part of the lacinia is concave on the dorsal aspect. In Melanoplus and Tettix, the mesoproximal angle is produced into a cone-shaped elevation. The suture between the lacinia and the stipes is obsolete on the dorsal aspect, but distinct and complete on the ventral aspect in all except Mantis, where it is interrupted, and Melanoplus, where it is represented by a furrow. An oblique suture on the ventral aspect of the stipes extends caudolaterad from the mesodistal angle in Gryllus, Orchelimum, and Anisolabis, and a mere indication of it occurs in Stenopelmatus and Tettix. Directed caudolaterad from the lateroproximal corner of the lacinia, there is another suture in Mantis, Gryllus, Orchelimum, and Melanoplus and slightly indicated in Diapheromera. The maxadentes are two in number, except in Melanoplus, which has three, and Tettix (fig. 99[1]) which has four, arranged in a transverse row. In Diapheromera, the single dens may be a product of fusion of two or three dentes. There is a non-dentate hamadens (*h*) on the mesal margin near the maxadentes in Gryllus, Orchelimum, and Stenopelmatus. The lacinarastrae are distinctly developed in all except Melanoplus and Tettix. In these genera, however, there are a number of long distinct setae (*rs*) on the mesal and ventral aspects. They are small and few in number in Diapheromera.

The galea is always two-segmented, and the suture between the segments is distinct on the ventral aspect. This suture, in Diapheromera and Orchelimum, is obsolete on the dorsal aspect. The proxagalea is short, transverse, and subcylindrical in Gryllus, Orchelimum, Stenopelmatus, and Anisolabis; is short and deeply concave on the mesal aspect in Mantis, and flattened, membranous, and slightly concave on the mesal half of the dorsal aspect in Melanoplus and Tettix, with the distal part dilated in the former and obtusely pointed in the latter. The distal end of the galea, except in Melanoplus, is membranous and provided with minute setae. The suture between the stipes and the proxagalea is obsolete on the ventral aspect in Mantis, Dia-

pheromera, Stenopelmatus, Melanoplus, and Tettix, and distinct on the dorsal aspect in Gryllus, Melanoplus, and Tettix. Crampton ('16) has named a small secondary lobe on the lateral margin of the distagalea of Diapheromera, the 'galealobulus.'

The maxillary palpus contains five segments. The proximal segment is small and cylindrical, with the distal portion frequently thickened; the second is larger than the first, except in Diapheromera, Tettix, and Anisolabis; the third is long and cylindrical—in Mantis the longest segment of the five; the third and fourth are usually subequal in length; and the fifth is clavate, subequal to the fourth in length in Gryllus, Orchelimum, Stenopelmatus, Melanoplus, Tettix, and Anisolabis. The distal segment in Mantis is small, cone-shaped, and subequal in length to the second; the largest and flattened in Diapheromera; and the tip provided with a small distinct papilla in Anisolabis. The two distal segments in Tettix are somewhat flattened. Excepting Mantis and Diapheromera, the distal end of the fifth segment of the palpus is covered with numerous setae.

The maxillae articulate with the head at the precoilae, dorsad of which the entoparartes always extend. On the dorsal aspect the margins of the cardo and stipes are continuous with the maxacoria and on the ventral with the labacoria.

The labium (*lb*) in Blatta (figs. 120 and 128) consists of the submentum (*sm*), mentum (*m*), and ligula (*li*) which includes the stipulae (*sp*), glossae (*go*), paraglossae (*pgo*), palpigers (*pp*), and labial palpi (*lp*). The submentum is a large subquadrate basal sclerite. Its caudal margin is transversely emarginate and is continuous with the microcoria (*ma*). The cephalic margin has a deep round emargination into which the semicircular mentum fits. The lateral margins of the submentum are folded over on to the dorsal aspect and form the lateral lobes (*ll*), the margins of which are continuous with the labicoria. The mentum is much smaller and narrower than the submentum, and the distal portion is only slightly chitinized. The suture between submentum and mentum is sometimes obsolete at the middle. The distal margin of the mentum is membranous and is folded dorsad and then caudad so that the ligula is placed at a

higher level and overlaps this portion of the mentum. The stipulae are distinct, quadrate, and bear a palpiger on each lateral aspect. The suture between each stipula and palpiger is distinct for a short distance on the ventral aspect and obsolete on the dorsal. The stipulae, except the fundarima, are membranous on the dorsal aspect. The fundarima (*fr*) is chitinized and distinct for some distance on the ventral aspect. The small, elongated, triangular appendage which is obliquely attached to the mesocephalic margin of each stipula is a glossa. Its distal end is covered with fine setae. The cephalic lobe, laterad of and separated by a distinct latarima (*lr*) from each glossa, is a paraglossa. The suture between the stipula and paraglossa is distinct. On the dorsal aspect the glossa and paraglossa are fused to the cephalic margin of the stipula without the indication of a suture. The proximal part of the mesal margin of each paraglossa is concave and the mesodistal portion is dilated and covered with minute setae. The lateroproximal portions of the glossae fit into the concavities of the paraglossae. The labial palpi are three-segmented and geniculate. The distal portion of the third segment is hemispherical and covered with minute setae. According to the interpretation of Crampton ('17), the labium articulates with the maxillariae. If so, this articulation is only slightly or not at all differentiated. The caudal angles of the submentum ordinarily are continuous with the microcoria.

The submentum in Mantis (figs. 121 and 129) is elongated, and narrowed toward the cephalic margin. In Diapheromera (figs. 127 and 134) it is considerably elongated and is deeply emarginate on the caudal margin. The condition in Gryllus (figs. 122 and 130) is similar to that in Blatta. It is wider than long in Orchelimum (figs. 125 and 133) and Stenopelmatus (figs. 123 and 131) and its caudal margin in the former is roundly emarginate. In Melanoplus (figs. 124 and 135) and Tettix (figs. 126 and 132) it is crescentic, and its caudal margin is deeply emarginate. The submentum of Anisolabis (figs. 136 and 137) is large, strongly chitinized, and subquadrate, with the cephalic and caudal margins only slightly emarginate. There is a nar-

row transverse sclerite (*ch*) caudad of the submentum. This piece, which is separated from the submentum by a distinct suture, has been designated as the 'submentum' by Packard ('83) and by practically all other writers, who have considered the large sclerite cephalad of it as the mentum and which is here designated as the submentum. This so-called 'submentum' is one of the sclerites of the microthorax. It is interesting to note that Hansen ('94) found a similar piece in Hemimerus, and the labium, according to his figures, is very similar to that of Anisolabis. In Diapheromera the caudal two-thirds of the submentum is membranous except for a narrow area along each lateral margin and a variable ovate area on the meson. The suture between submentum and mentum is distinct and complete, except in Melanoplus where the mesal portion is obsolete. The lateral lobes are distinct, except in Melanoplus and Tettix; they are best developed in Anisolabis.

The mentum is narrow, transverse, and distinctly smaller than the submentum in Diapheromera, Gryllus, Stenopelmatus, and Anisolabis; is more or less distinct in Melanoplus and Tettix; and is indistinct in Mantis and Orchelimum, where it is fused with the stipulae without indication of sutures. The caudal margin of the mentum in Orchelimum, Stenopelmatus, Melanoplus, and Tettix slightly overlaps the cephalic margin of the submentum and is at a higher level than the latter.

The stipulae are subquadrate and distinct in all except those where the mentum has fused with the stipulae. They are elongated in all except Gryllus and Melanoplus, where they are transverse. The mesarima is very deep in Mantis; moderately deep in Orchelimum and Melanoplus, and reaches the cephalic margin of the mentum in Anisolabis. The fundarima extends caudad as a chitinized thickening in Diapheromera, Gryllus, Orchelimum, Stenopelmatus, and Tettix. In the last four genera, the caudal end is connected with thickenings which extend laterad. The suture which separates each stipula from a glossa and paraglossa is complete in Gryllus, Melanoplus, Tettix, Anisolabis, and incomplete in the others. It is entirely wanting on the dorsal surface.

The palpiger is small and lateral in position in all except Melanoplus, where it is located on the ventral aspect. The suture between the palpiger and stipula of each side is obsolete or indistinct in Mantis, Diapheromera, Orchelimum, Tettix, and Anisolabis.

The glossae are small and e'ongate. In Gryllus, Orchelimum, Stenopelmatus, and Tettix, they are pointed, in the remaining genera more or less rounded. In Melanoplus the dextral glossa is distinctly larger than the sinistral, which is rudimentary.

The paraglossa are very mugh larger than the glossae, except in Mantis, where they are slightly smaller. In Melanoplus they are enormously expanded and decidedly larger than the glossae; are more or less flat in Mantis, Diapheromera, Melanoplus, and Tettix; thicker and folded mesad in Gryllus, Orchelimum, and Stenopelmatus. In all of the latter genera the mesal, cephalomesal, or dorsal portion of each paraglossa is concave and overlaps the glossa to a greater or less extent. In Gryllus and Stenopelmatus a furrow, some distance caudad of the suture, separates each paraglossa and stipula. This furrow may be an indication of the suture which separates the paraglossa into two segments—a condition comparable to the two-segmented galea of the maxilla. Anisolabis has only a single appendage attached to the distal end of each stipula. As to the interpretation of this, the literature is confusing. Fabricius (1776) characterized the labium of Forficula as 'trifidum,' but Olivier (1791) correctly spoke of the two equal lobes of the labium. Later writers seem to have noticed the bifurcated condition of the labium, but, with the exception of Borman ('00), have not recorded their opinion as to the homology of these lobes. This author states that the glossa and paraglossa have fused and formed the single distal lobe attached to each stipula, but I have been unable to find his 'deutliche Trenungslinie,' and there is no indication of the real nature of this appendage. It may represent the glossa, the paraglossa, or the product of the fusion of the two.

The labial palpi are invariably composed of three segments. The basal segment of each palpus is the smallest of the three except in Mantis, where all are subequal. The middle segment

is smaller than the distal one, except in Mantis and Anisolabis. The distal segment is long, cylindrical, clavate, and its hemispherical tip is covered with minute conical setae in Gryllus, Orchelimum, Stenopelmatus, and Melanoplus, and is more or less depressed in Diapheromera and Tettix. In Anisolabis the last segment is clavate and smaller than the second and its tip bears a small, cuticular papilla (fig. 137). The labial palpi are nearly straight in Mantis, but are more or less geniculate in other genera.

The prepharynx of generalized biting insects consists of a well-differentiated propharynx (*prx*), parapharynx (*ppx*), and ambipharynx (*ax*). The propharynx is dorsal in position and includes the epipharynx (*ex*), a pair of tormae (*tm*), and the epigusta (*eg*). The parapharynx is ventral in position, is linguiform, and consists of two parts, the proximal basipharynx (*bx*) and the distal hypopharynx (*hx*). The former includes the pharyngeae (*prg*), the paralinguae (*pln*) with the linguacutae (*lg*) which bears the linguatendons (*lg*), the lingulae (*ln*), and the subgusta (*su*); while the latter includes a pair of saliviae (*sl*), which support the salivos (*so*), and the ventral membranous oscula (*ox*). That part of the pharynx caudad of the prepharynx is the postpharynx (*pox*). It is well to remember that the parapharynx corresponds approximately to the 'hypopharynx' of most writers.

The prepharynx of Blatta (figs. 151, 152, 153, and 154) includes all the parts enumerated above. The epipharynx is the same in size and shape as the labrum and the surface is concave near the cephalic margin. The tormae are the distinct, brownish, X-shaped sclerites. One arm of the X extends to the laterocaudal angle of the labrum, forming on the dorsal aspect, a distinct landmark for the lateral end of the clypeolabral suture and another arm extends caudad for a short distance, where it disappears from the surface, becoming an ental bar to which the principal retractor muscles of the labrum are attached. The other two arms of each torma extend mesad and enclose a clear membranous circular area which is provided with minute sensory pits. A brownish claw-shaped chitinized area, extending

cephalad from the cephalic margin of each circular area, bears about fifteen, short, conspicuous, subdecumbent, spine-like setae. The surface of the epipharynx between the two brownish areas and the area cephalad of them is densely and uniformly covered with short fine spinulae, the majority of which are directed more or less mesocaudad. The cephalic emargination of the epipharynx is beset with stiff, brownish spinulae. The epigusta is flat or slightly concave, and is uniformly covered with short fine spinulae or solid 'hairs.' The ambipharnyx is rather broad, smooth, and thinner than the adjoining coriae.

The parapharynx is linguiform and its various parts are well differentiated. The pharyngea is the long, slender sclerite along each side of the subgusta. Its caudal portion is expanded, less chitinized, and extends dorsocaudad, supporting the lateral wall of the postpharynx. The entrance to the latter is located, therefore, near the middle of the pharyngeae. The paralingua is the convex subquadrate sclerite fused to the cephalic end of each pharyngea and extends caudoventrad. A long, narrow, crescentric sclerite, the linguacuta, lies near the caudal angle of each paralingua. It extends for a short distance dorso-caudolaterad and merges into a slender ental tendon, the linguatendon, which, after passing over the dorsal side of the rectotendon of the mandible, extends into and toward the lateral side of this structure. Mangan ('08) figured, for the first time, this tendon in Periplaneta australasiae. The lingula is the subcrescentic brownish sclerite fused to the cephalic end of and dorsad to each paralingua. It is sparsely covered with short, distinct, spine-like setae. The mesal end of the lingula is produced meso-entad and approaches the one on the other side, but remains separate on the meson. This part of the lingulae produces the constriction of the propharynx between the basipharynx and hypopharynx. The membranous area caudad of the mesal arms of the lingulae is convex and slightly elevated, forming a ridge which is provided on each side with a band of brownish spinulae. Bugnion ('16) erroneously designated and figured this ridge in Blatta americana as 'l'entrée du pharynx.' There is a group of small distinct sensory pits on each side and slightly cephalad of the

median ridge. The subgusta is broad, membranous, and provided with a shallow, median, longitudinal furrow. The hypopharynx is the subquadrate structure cephalad of the constriction of the parapharynx. Its dorsal surface is convex, slightly elevated on the meson, and is densely covered with spinulae. The distal end of hypopharynx is also spinulate. There is an elongated, triangular, slightly chitinized area cephalad of and indefinitely separated from each lingula. The salivia is the large lanceolate sclerite, ventrad of the cephalic part of the lingula and the triangular area cephalad of it. It extends cephalodorsad to the caudal margin of the distal spinulate area. Its caudal third is strongly chitinized, its caudal third moderately, and its middle third only slightly. This sclerite bears several spine-like setae. The ventrocaudal angle of each salivia is rounded and produced into a chitinized, tapering bar which extends ventro-caudo-mesad and meets the one from the other side, forming a deep semicircular structure. The salivos is located cephalad of this median structure. Each ventrolateral angle of the hypopharynx, ventrad of the salivia and cephalad of the bars supporting the salivos, is obtusely rounded and moderately chitinized. The ventromeson of the hypopharynx is concave, the concavity widens caudad, and is provided with a shallow median furrow. On each side and cephalad of the salivos is a pit leading into a short blind pouch which Mangan ('08) has suggested to be a 'salivary receptacle,' but its function has not been determined. The oscula is the membranous area located ventrad of the hypopharynx. It extends laterad and then dorsocaudad and is continuous with the labacoria and lingula. The salivos is protected by a portion of the oscula which extends ventrocephalad of the salivial bars. The salivary duct is large and extends caudad for some distance before bifurcating.

The general plan of the organization of the prepharynx in all the genera studied manifests a striking similarity, and the homology of each component part is demonstrable with a fair degree of certainty. The differences in size, shape, and position in these structures, though great in some cases, are reducible in general to a gradual series of modification.

The epipharynx is setiferous, membranous, and usually concave and is similar in size and shape to the labrum. The surface bears setae and spinulae which are local in their distribution in Gryllus and others. There are often chitinized structures which are usually more distinct on the ental than on the ectal surface and which may be Y-shaped as in Melanoplus or wedge-shaped as in Gryllus.

The tormae are always present, distinct, well chitinized, and twisted, and bear definite relation to the clypeolabral suture. The dorsal arm of the twisted body is attached to the lateral angle of the suture and the ventral arm to the caudolateral angle of the epipharynx, while the body of each torma connects the two arms. There is a variation in size, shape, and complexity from the simple type found in Stenopelmatus to the many-branched type of Gryllus. The mesal ends of the tormae are sometimes connected by a thickening (fig. 159).

The epigusta is membranous and often includes a few tendinous thickenings. It is frequently spinulate and bears thickenings and sensory pits. It gradually merges with the postpharynx and ambipharynx; there is never a sharp line of demarcation between them. The epigusta is never extensive.

The ambipharynx is membranous and not well differentiated. It is usually restricted by the encroachment of the mandibles. In Gryllus it is spinulate near the entrance to the postpharynx and bears a small chitinized area cephalad of each mandible.

The parapharynx is well developed and, since it is complex in organization and since there is no adequate description published, it will be described with more or less detail. The distribution and localization of the setae and spinulae on the chitinized and membranous portions of the parapharynx differ considerably. The asymmetry of the surface structures observed in many genera (Gryllus, Tettix, Anisolabis, and others) is due to the adjustment necessary to secure the close fitting of different elements of the mouth-parts, both when in repose and in use.

In Mantis (fig. 161) the parapharynx resembles that of the cockroach. The pharyngea is distinct, slightly chitinized, and extends caudad along the ventrolateral margin of the subgusta

into the lateral wall of the postpharynx. The caudal end is on the dorsolateral margin of the postpharynx and supports its dorsal and lateral walls. The paralingua is an inverted Y-shaped sclerite on the lateral aspect cephalad of and fused with each pharyngea. To the strongly chitinized dorsal stem of the Y a prominent linguatendon is attached. It extends laterad, and, after passing over the rectotendon of the mandible, enters this structure. The caudal portion of the subgusta is broad and the cephalic is constricted between the two paralinguae. The lingula is the distinct setiferous sclerite fused by a narrow neck to the cephalic end of each paralingua. The dorsal surface of the parapharynx is constricted between the two lingulae. The salivia is a large chitinized area occupying the ventral half of each lateral aspect of the hypopharynx. Its caudal angle is produced into an arm which supports the salivos on the ventromeson. A slightly chitinized area lies cephalad of each lingula and dorsad of each salivia. The ventral aspect of the hypopharynx is concave and furrowed on the meson and the chitinized extensions of the saliviae occupy the caudolateral portions. The salivos is protected on the ventral side by a thick triangular membrane. The salivary duct is very small and extends caudad for some distance before bifurcating. The oscula is narrow and indefinite and the salivos is located dorsad of the cephalic part of the mentum.

The parapharynx in Diapheromera (fig. 163) is compressed and boot-shaped. The pharyngea is a large irregular sclerite extending into the lateral and ventral walls of the postpharynx. Its cephalic end terminates indefinitely in the membrane laterad of the subgusta. The subgusta is deeply furrowed on the meson, and each caudolateral half is strongly chitinized and fused to the pharyngea without indication of a suture. The paralingua is the ax-shaped sclerite on each lateral aspect of the subgusta. Its dorsocaudal angle is pointed, the caudoventral bifurcated, and the linguatendon is attached to its laterodorsal arm. The cephalodorsal angle is more chitinized than the other parts and curves cephalomesad, while the opposite angle extends cephaloventrad and meets the dorsal extension from the caudal part

of the salivia. The lingula is the indefinite, but more chitinized, area cephalad of each paralingua. The two lingulae fuse on the meson without indication of a suture, and their dorsocaudal margin is thickened and constricts the parapharynx. The greater part of each lingula lies cephalad of this constriction instead of caudad of it as in the other genera. The parapharynx caudad of this constriction is decidedly elongated on account of the increase in the size of the mandibles. The salivia is uniformly chitinized and occupies the entire ventral half of each caudo-lateral aspect and the greater part of the ventral aspect of the hypopharynx. Its caudoventral angle is sharply produced and the mesal portion extends caudad as a broad tube and supports the salivos. The ventral surface cephalad of the salivos is submembranous and convex. The salivary duct is small and bifurcates immediately caudad of the salivos. The oscula is narrow on the sides and membranous. The modifications of the mouth-parts due to the cephalization of the mouth subsequent to the elongation of the head have produced the peculiarities of the parapharynx noted above.

In Gryllus (fig. 157) the parapharynx is large and linguiform. The pharyngea is a distinct L-shaped sclerite on each caudolateral aspect of the parapharynx. One arm of the L is vertical and is produced into a thinner extension which extends caudad along the lateral margin of the subgusta and, on reaching the postpharynx, is bent dorsad and extends along its lateral wall. A small, but distinct, chitinized pocket, immediately ventrad of the horizontal arm of the L, may be the homologue of the cuticular pouch near the same position in Blatta. The paralingua is a brownish, subquadrate, non-setiferous sclerite cephalad of each pharyngea. Its cephaloventral margin is thickened and reflected slightly entad. Each lingula consists of two parts, an ectal and an ental. The ental part is thick, short, strongly chitinized and curved toward the meson. The sinistral arm is slightly caudad of the dextral. The ectal part of each lingula is convex, setiferous, triangular, and chitinized, and is subdivided by a short suture-like furrow which defines the less chitinized convex area on the dorsal side from the distinct larger area on

the ventral. The caudal portion of the subgusta is flattened, the cephalic portion is convex, and there is a constriction near the middle. Laterocephalad of each lingula is a less chitinized triangular area similar to the one in Blatta. The tendency to asymmetry occurs in the ental arms of the lingula, in the elliptical area cephalad of them, and in the patches of brownish spinulae. Those on the dextral side are more advanced in position and more conspicuous than the sinistral. The salivia is a large, distinct, triangular sclerite cephaloventrad of each lingula and ventrad of the triangular area mentioned above. The apex of the triangular salivia is produced ventromesad into a long pointed extension which, on reaching the meson, supports the salivos. The ventral surface of the hypopharynx is membranous and broadly and deeply folded. The middle portion of the membrane is folded over itself and forms a deep pocket into which the distal portion is retracted. This retractile portion is covered with pseudotrachea-like thickenings which extend from the main mesal trunk to the sides in an oblique parallel manner. These smaller thickenings unite on the lateral margin into a large lateral trunk which converges cephalad and extends over the distal margin onto the dorsal surface of the hypopharynx. This pseudotracheated portion may be evaginated and protruded much further cephalad than the distal margin of the labium. Figure 157 represents this portion slightly out of place. The oscula is narrow cephalad of the salivos, deeply folded around the ventral margin of the salivia, and is produced on each lateral aspect where it merges into the labicoria.

In Orchelimum (fig. 158) the parapharynx is well developed and linguiform. The pharyngea is long, slender, slightly chitinized, and extends along each side of the subgusta and then obliquely dorsad into the lateral wall of the postpharynx. The paralingua is the ax-shaped sclerite fused to the cephalic end of each pharyngea. Its ventrocaudal arm is directed mesad and then laterad and is connected with the mandible by a poorly differentiated linguacuta. The lingula is distinct and fused to the dorsocephalic angle of each paralingua; a mesal extension from its dorsal margin constricts the parapharynx at this point.

The subgusta is slightly concave and is continuous with the broad ventral wall of the postpharynx. The cephalic ends of the paralinguae constrict the subgusta. There is a narrow, distinctly setiferous, moderately chitinized area cephalad of each lingula corresponding to that in Blatta. The salivia is large and chitinized. A nearly vertical arm extends dorsad from it into the setiferous area above. This may correspond to the similar arm in Gryllus. The cephalic part of each salivia is longitudinally convex and forms a shelf along the lateroventral margin of the hypopharynx. The ventral aspect of the hypopharynx is membranous and longitudinally furrowed on the meson. The caudolateral angles are strengthened by the chitinized part of the saliviae. The oscula is narrow and the salivos is dorsad and slightly cephalad of the middle of the mentum.

The parapharynx in Stenopelmatus (fig. 159) is large, quadrate, and linguiform. The pharyngea is a long slender sclerite along each side of the subgusta. Its expanded caudal part supports the lateroventral margin of the postpharynx. The cephalic half is uniformly narrow and extends cephaloventrad. The distinct brownish sclerite located on each lateral aspect, cephalad of the pharyngea, is the paralingua. A membranous linguacuta is attached to the caudoventral angle of each paralingua. It extends laterad toward the rectotendon of the mandible. The lingula is the large triangular sclerite cephalad of each paralingua; its dorsocephalic angle is produced into a curved ental arm which approaches the arm on the other side. The caudal part of the subgusta is wide and flat, and the cephalic part converges narrowly. The salivia is located along the ventrolateral margin of the hypopharynx. Its caudal part, ventrad of each lingula, is a distinct bar which extends nearly horizontally to the ventro-caudolateral angle of the hypopharynx and then bends mesocaudad to the wide hooded salivos. The distal part of the salivia is longitudinally convex so as to form a shallow shelf on each ventrolateral margin of the hypopharynx. The dorsal aspect of the hypopharynx is distinctly bent near its middle. The oscula cephalad of the salivos is very much narrowed by the chitinized area caudad of the mesarima of the

labium. The apex of the triangular area is applied against the laterocaudal angle of the salivia. The oscula on the lateral aspect forms a small membranous fold at the proximal end of the hypopharynx.

In Melanoplus (figs. 156 and 162) the parapharynx is subglobular and obtusely pointed at the distal end. The pharyngea is long and slender, its caudal part extending along the ventrolateral margin of the postpharynx; the cephalic part is strongly chitinized. The subgusta is deeply furrowed on the meson, and constricted between the cephalic ends of the pharyngeae. The paralingua is the small V-shaped sclerite ventrad of and fused with the cephalic part of each pharyngea. The ventral arm of the V extends ventrad and then caudolaterad into the poorly differentiated linguacuta which is attached to the mesocephalic end of the rectotendon of each mandible. The lingula is the brownish subquadrate sclerite located slightly ventrad and cephalad of the cephalic end of each paralingua. Its cephalic margin is expanded and its mesocephalic end bent mesad. The hypopharynx is narrowest between the distal ends of the lingulae and is produced laterad and strongly convexly cephalad of this constriction. There is a constriction near the distal third of the hypopharynx where the lateral surface is produced like a shoulder. This prominence caudad of the distal constriction was interpreted by Folsom ('00) as a rudimentary 'superlingua.' The salivia consists of two subdivisions. The dorsal is a long strongly chitinized sclerite extending obliquely from the dorsal constriction along the ventral margin of the proximal part of the hypopharynx. The other is a large chitinized plate on the lateral and ventral aspects. The ventral part is irregular and is continuous with the dorsal part, with a faint suture-like furrow between them. It extends mesad to form a support for the salivos. The ventral aspect of the hypopharynx is distinctly convex and is produced into a small pocket under the salivos. The salivary duct is slightly dilated and depressed immediately caudad of the salivos. It extends caudad for a short distance, then bifurcates. The oscula is flat under the free part of the hypopharynx, protects the salivos, and extends laterad, then caudad, to the lingulae and paralinguae where it is continuous with the labicoria.

The parapharynx in Tettix (fig. 160), somewhat similar to that of Melanoplus, is well developed and linguiform. The pharyngea is the short distinct sclerite located at each caudolateral angle of the subgusta. Its main part is on the ventral side of the lateral wall of the postpharynx. Fused to the end of each pharynx is the long curved paralingual sclerite. The connection between these is weaker and less distinct on the right side than on the left. The ventral arm of the paralingua curves ventrad and merges into the linguacuta. The strongly chitinized crescentic sclerite fused to each paralingua is the lingula. The greater part of it is on the ental surface and extends mesad. The subgusta is longitudinally concave and is constricted between the paralinguae. The position of the well-defined subtriangular chitinized area cephalad of each lingula suggests that it is homologous with the long oblique part of the salivia of Melanoplus. The larger chitinized sclerite on the ventrolateral aspect of the hypopharynx is the salivia and is separated from the triangular area on the dorsal side by an oblique suture. An oblique thickening near its caudodorsal part has its ventral end produced into a prominent blunt extension, probably homologous with that in Stenopelmatus. The mesal extension of the salivia is heavily chitinized and nearly transverse. The oscula is more or less flat cephalad of the salivos. The salivary duct bifurcates immediately caudad of the salivos, the latter being dorsad of the caudal margin of the mentum. There is an asymmetry in that the right mesal arm of the lingula is slightly caudad of the left one and the adjacent large patch of spinulae is much more extensive on the right side than on the left.

The parapharynx in Anisolabis (fig. 138) has its own characteristics, but in the main conforms to the orthopteran type. It is well developed, broad, and trilobed at the distal end. A long pharyngea lies each side of the subgusta. Its caudal half is expanded and extends obliquely dorsad into the lateral wall of the postpharynx. Its cephalic half is narrow and strongly chitinized. The oblique L-shaped sclerite fused to the cephalic end of each pharyngea is the paralingua. The linguatendon is fused to the ventral, wider, less chitinized arm of the paralingua

and extends toward the rectotendon of the mandible. The prominent X-shaped sclerite cephalad of each paralingua is the lingula, the lateral aspect of which is concave and is produced into a strongly chitinized caudal arm and a narrow arm which is reflected ventrocephalad. The transverse arm of each lingula apparently fuses on the meson with the one on the other side. A narrow semicircular projection, extending caudad from the mesal end of each transverse arm, touches the cephalic end of the paralingua and a prominent mesal spinulate ridge caudad of the lingulae. The subgusta is concave and carinate as noted above. The distal end of the hypopharynx is trilobed. The median lobe is large, symmetrically triangular, and its apex is pointed. Each lateral lobe is concave on its mesal aspect and overlaps the lateral margin of the proximal part of the median lobe. On the ventral two-thirds of each lateral aspect of the hypopharynx is a large, moderately chitinized, convex sclerite, the salivia. Its caudal angle is produced into a mesal arm supporting the salivos on the ventromeson. The ventral aspect of the hypopharynx is deeply concave near the proximal portion, but the median third is notably convex, while the distal third is membranous. A narrow chitinized area extends cephalad along the lateral margin of the hypopharynx and supports the ventromesal part of the lateral lobe. The mesal arms of the saliviae form on the ventromeson a cone-shaped structure whose apex is directed caudad and whose cephalic margin is arched over by a narrow transverse bar which is provided with a small median notch. The cone is fused solidly to the strongly chitinized V-shaped vertical frame which arises from the keel-like ental ridge at the base of the mesarima of the labium. The salivary duct is moderately large and attached to the caudal end of the cone and its opening is at the median notch of the transverse bar. There is a distinct asymmetry of parts on the dorsal aspect of the hypopharynx. The dextral ligula is straighter and narrower than the sinistral, but its cephalic process on the meson is very much larger than the sinistral; the right paralingua is slightly in advance of the left, and the sinistral lateral lobe of the hypopharynx is smaller and overlaps the median lobe to a

less extent than the dextral. There is also a difference in the number of extensions on the epigusta. All these peculiarities seem to be of secondary importance, since their presence can be explained by the asymmetrical conditions of the mandibles which, when brought together, leave an irregular space for the parapharynx to fill. The nature of the lateral lobes is more difficult to explain. They are the 'paraglossae' of the older writers, and correspond to the 'maxillulae' of Hansen ('94) and 'superlingue' of Folsom ('00). Berlese ('06) considers the entire parapharynx, including the lateral lobes, as derived from the labium, and thus disagrees, like many others, with Folsom's view concerning the origin and nature of these lobes of the hypopharynx. Carpenter ('16) figures the labium of Isolepisma bisetosa with a 'maxillula' which simulates the conditions found in Orthoptera and Euplexoptera.

SUMMARY

1. This study on the comparative anatomy of the head and mouth-parts of orthopteran insects has resulted in establishing the homology of all of the structures present in generalized and specialized Orthoptera, and in elucidating the hitherto but little studied structures, the propharynx and tentorium. Excepting variations in size, shape, and other superficial differences, not of primary importance, all of the structures examined reveal a remarkable uniformity in their general organization and at the same time indicate the trend of evolutionary processes which are responsible for the apparent gradation in specialization manifested by these structures.

2. Since morphology offers fundamental evidence for the determination of phylogeny, a word may be added, with due appreciation of the limitation of the data at hand, as to the probable genealogical relations of the insects under consideration. Briefly stated, the anatomical evidences presented in this paper show that the Blattidae is the most generalized; that the Mantidae is closely related to the Blattidae, that the Gryllidae follows the Mantidae, although it is not likely to have developed directly

through the latter; that the Locustidae and Acridiidae come next in the order named, and that the Phasmidae appears to be most remote from the Blattidae, although its exact phylogenetic position cannot be determined. The Forficulidae possess evidences of orthopteran affinity in their general morphological organization, but differ from the Orthoptera in the character of the maxillae, parapharynx, and labium.

The structures dealt with in this study are summarized as follows:

3. The exoskeleton of the head consists of vertex, front, clypeus, labrum (preclypeus and postclypeus), mandibulariae, occiput, postgenae, and maxillariae. The vertex includes the genae and bears the compound eyes, ocelli, antacoriae, and antennariae. The occipital foramen is bounded by the occiput, postgenae, and maxillariae.

4. The endoskeleton of the head:

BODY	ARMS	EXTERNAL MARKS	ASSOCIATED WITH
Corpotentorium	Metatentorium	Metatentorina	Postgena
Laminatentorium	Pretentorium	Pretentorina	Front, clypeus, and mandibularia
	Supratentorium	Supratentorina	Antennaria

5. The movable parts of the head:

STRUCTURE	BORNE ON SCLERITE	INCLUDING
Antennae	Antennariae and antacoriae	Scape, pedicel, flagellum, antatendons
Mandibles	Mandibulariae	Dentes, mola, acia (Blatta), brustia, artes, tendons
Maxillae	Maxillariae	Cardo (alacardo and subcardo), pararates, stipes, subgalea, palpifer, palpi, lacinia, galea (proxigalea and distagalea), maxacoria
Labium	Microcoria	Submentum (lateral lobes sometimes), mentum, ligula, labicoria. Ligula includes stipula, palpiger, palpus, glossa, paraglossa (dista- and proxa-)

6. The parts of the prepharynx:

- Pharynx
  - Postpharynx
  - Prepharynx
    - Propharynx
      - Epipharynx — Tormae
      - Epigusta
    - Ambipharynx
      - Subgusta
      - Pharyngeae
    - Parapharynx
      - Basipharynx — Paralinguae (linguacutae, linguatendons)
      - Lingulae
      - Hypopharynx
        - Saliviae
        - Salivos (salivary duct)
        - Oscula

7. The articulation of the appendages:

APPENDAGE	ARTICULATING PIECE	ARTES	COILA	TENDON
Antennae	Scape	Antartes or base of scape	Antennaria	Antatendons
Mandibles	Proximal part of mandible	Preartis	Precoila on clypeus	Extensotendon
		Postartis	Postcoila on postgena	Rectotendon
Maxillae	Subcardo	Parartis		
		Exparartis	Paracoila on maxillaria	Postmaxatendon
		Entoparartis		Premaxatendon
Labium	Submentum (associated with microcoria)			

8. The sutures of the head:

SUTURE	LOCATION	BOUNDARY BETWEEN
Epicranial		
Stem	Epicranium	Two halves of vertex
Arms	Epicranium	Vertex and front
Frontogenal	Front	Front and gena
Frontoclypeal	Front	Front and clypeus
Clypeal	Clypeus	Preclypeus and postclypeus
Clypeolabral	Clypeus	Clypeus and labrum
Occipital	Postgena	Vertex and postgena
Mandogenal	Mandibularia	Mandibularia, front, and gena

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PLATES

LIST OF ABBREVIATIONS

a, antenna
aa, antacoila
ac, acia
ad, antartis
al, alacardo
an, antacoria
ar, antennaria
at, antatendon
ax, ambipharynx
b, brustia
bx, basipharynx
c, clypeus
ca, cardo
ce, compound eye
ch, chitinized area
cls, clypeolabral suture
cr, crassa
cs, clypeal suture
ct, corpotentorium
d, dentes
dd, distadentes
dg, distagalea
ea, epicranial arm
ec, extensacuta
eg, epigusta
en, entoparartis
es, epicranial stem
et, extensotendon
ex, epipharynx
ey, exparartis
f, front
fcs, frontoclypeal suture
fgs, frontogenal suture
fl, flagellum
fr, fundarima
g, gena
gl, galea
go, glossa
h, hamadens
hx, hypopharynx
l, labrum
la, lacinia
lc, labicoria
lg, linguatendon or linguacuta
ll, lateral lobe of submentum
ln, lingula
lo, lateral ocellus
lp, labial palpus
lr, latarima
lt, laminatentorium
lx, lateral lobe of ligula
m, mentum
ma, microcoria
mb, mandibularia
mc, maxacoria
md, mandible
mi, muscle impression
ml, mola
mgs, mandogenal suture
mn, metatentorina
mo, median ocellus
mp, maxillary palpus
mr, mesarima
ms, maxadentes
mt, metatentorium
mx, maxilla
my, maxillaria
oc, occiput
od, odontoidea
of, occipital foramen
ol, oculata
os, occipital suture
ot, oesotendon
ox, oscula
p, pedicel
pd, proxadentes
pf, palpifer
pg, proxagalea
pgo, paraglossa
pgn, postgena
pl, paracoila
pln, paralingua
pm, parademe
pmt, premaxatendon
pn, pretentorina
pot, postmaxatendon
pox, postpharynx
pp, palpiger
pr, precoila
prg, pharyngea
pt, pretentorium
ptc, postartis
ptl, postcoila
py, preartis
rc, rectacuta
rs, lacinarastra

rt, rectotendon
s, stipes
sa, subcardo
sb, scrobe
sc, scape
sg, subgalea
sl, salivia
sm, submentum
sn, supratentorina
so, salivos
sp, stipula
st, supratentorium
su, subgusta
td, tendon
tm, torma
v vertex

PLATE 1

EXPLANATION OF FIGURES

1 Blatta orientalis, dorsal aspect of head.
2 Blatta orientalis, articulation of mandible.
3 Mantis religiosa, male, dorsal aspect of head.
4 Mantis religiosa, female, dorsal aspect of head.
5 Gryllus pennsylvanicus, dorsal aspect of head.
6 Stenopelmatus sp., dorsal aspect of head.
7 Orchelimum vulgare, dorsal aspect of head.
8 Diapheromera femorata, dorsal aspect of head.
9 Mantis religiosa, female, lateral aspect of head.
10 Melanoplus differentialis, dorsal aspect of head.
11 Tettix arenosus, dorsal aspect of head.
12 Anisolabis maritima, dorsal aspect of head.
13 Blatta orientalis, lateral aspect of head.
14 Gryllus pennsylvanicus, lateral aspect of head.
15 Diapheromera femorata, lateral aspect of head.
16 Diapheromera femorata, articulation of mandible.
17 Anisolabis maritima, lateral aspect of head.
18 Stenopelmatus sp., lateral aspect of head.
19 Melanoplus differentialis, lateral aspect of head.
20 Tettix arenosus, lateral aspect of head.
21 Anisolabis maritima, lateral aspect of head, appendages removed.
22 Orchelimum vulgare, lateral aspect of head.

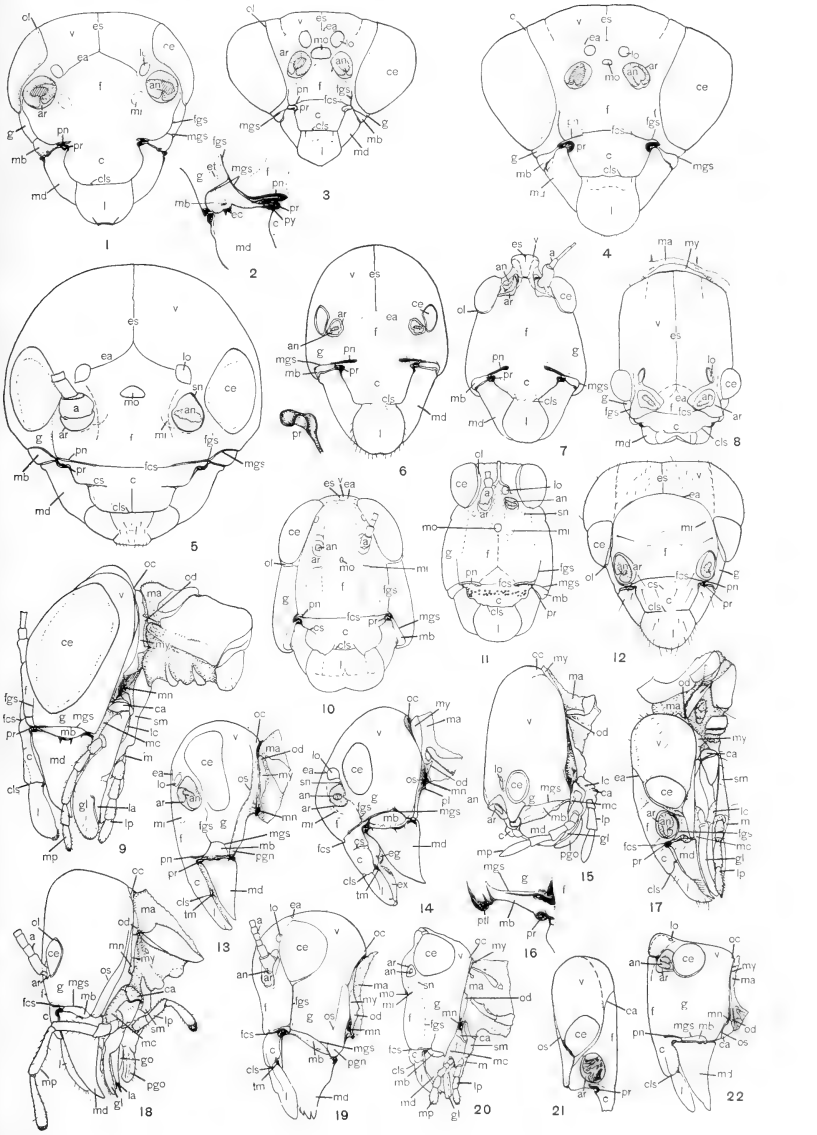

PLATE 2

EXPLANATION OF FIGURES

23 and 24, 27 to 32, 35 Ventral aspect of the head, appendages removed.

36 to 41 Endosekleton of the head, dorsal wall of the head capsule, appendages, and internal organs removed.

23 Blatta orientalis.
24 Mantis religiosa.
25 Diapheromera femorata, caudal aspect of the head, appendages removed.
26 Mantis religiosa, postcoila enlarged.
27 Orchelimum vulgare.
28 Gryllus pennsylvanicus.
29 Diapheromera femorata.
30 Melanoplus differentialis.
31 Stenopelmatus sp.
32 Anisolabis maritima.
33 Diapheromera femorata, cephalic aspect of the head, appendages in situ.
34 Melanoplus differentialis, postcoila enlarged.
35 Tettix arenosus.
36 Blatta orientalis.
37 Gryllus pennsylvanicus.
38 Diapheromera femorata.
39 Melanoplus differentialis.
40 Tettix arenosus.
41 Mantis religiosa.

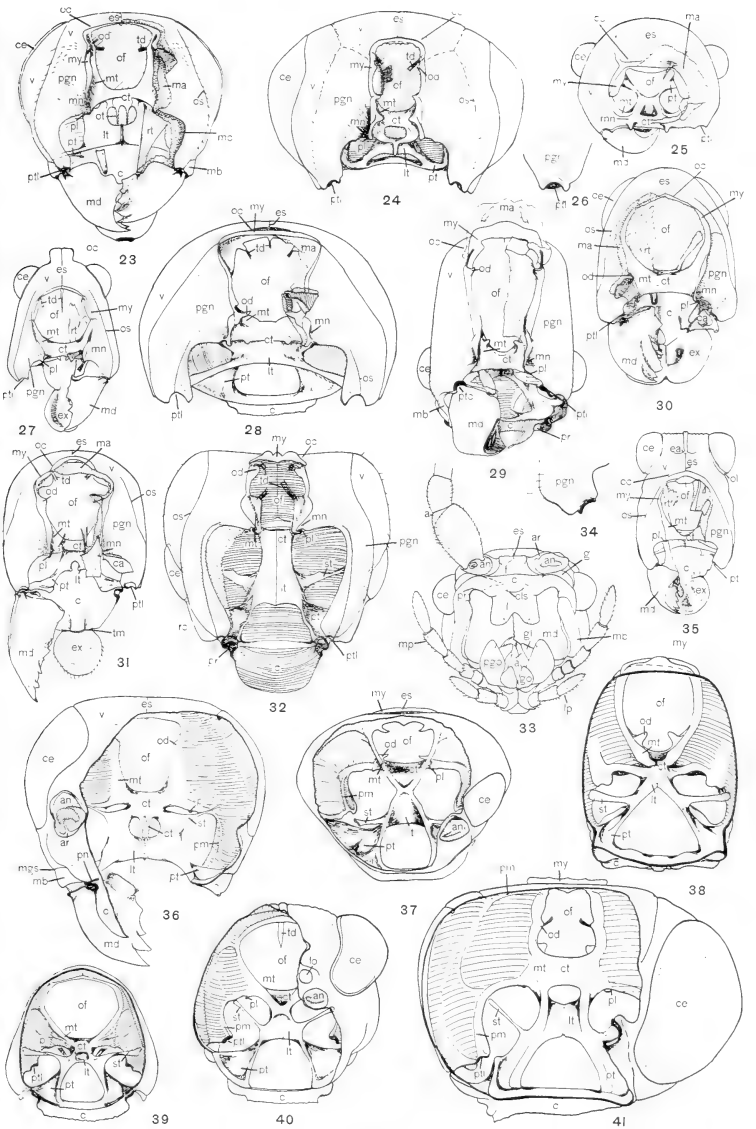
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PLATE 3

EXPLANATION OF FIGURES

42 to 44 Endoskeleton of the head, dorsal wall of the head capsule, appendages, and soft internal tissues removed.

45 to 53 Endoskeleton of the head in median longitudinal section, soft internal tissues removed.

54 to 69 Antacoria, antennaria, and proximal segments of the antennae.

42 Anisolabis maritima.
43 Stenopelmatus sp.
44 Orchelimum vulgare.
45 Blatta orientalis.
46 Mantis religiosa.
47 Gryllus pennsylvanicus.
48 Stenopelmatus sp.
49 Melanoplus differentialis.
50 Orchelimum vulgare.
51 Diapheromera femorata.
52 Tettix arenosus.
53 Anisolabis maritima.
54 Blatta orientalis, female, cephalic aspect.
55 Blatta orientalis, female, caudal aspect.
56 Blatta orientalis, male, cephalic aspect.
57 Mantis religiosa, cephalic aspect.
58 Mantis religiosa, caudal aspect.
59 Gryllus pennsylvanicus, cephalic aspect.
60 Gryllus pennsylvanicus, caudal aspect.
61 Anisolabis maritima, cephalic aspect.
62 Orchelimum vulgare, cephalic aspect.
63 Stenopelmatus sp., cephalic aspect.
64 Stenopelmatus sp., antennaria, antenna removed.
65 Orchelímum vulgare, antartis and antatendon.
66 Diapheromera femorata, portion of head capsule with proximal segments of antenna.
67 Melanoplus differentialis, ental aspect of antacoria.
68 Melanoplus differentialis, cephalic aspect.
69 Tettix arenosus, cephalic aspect.

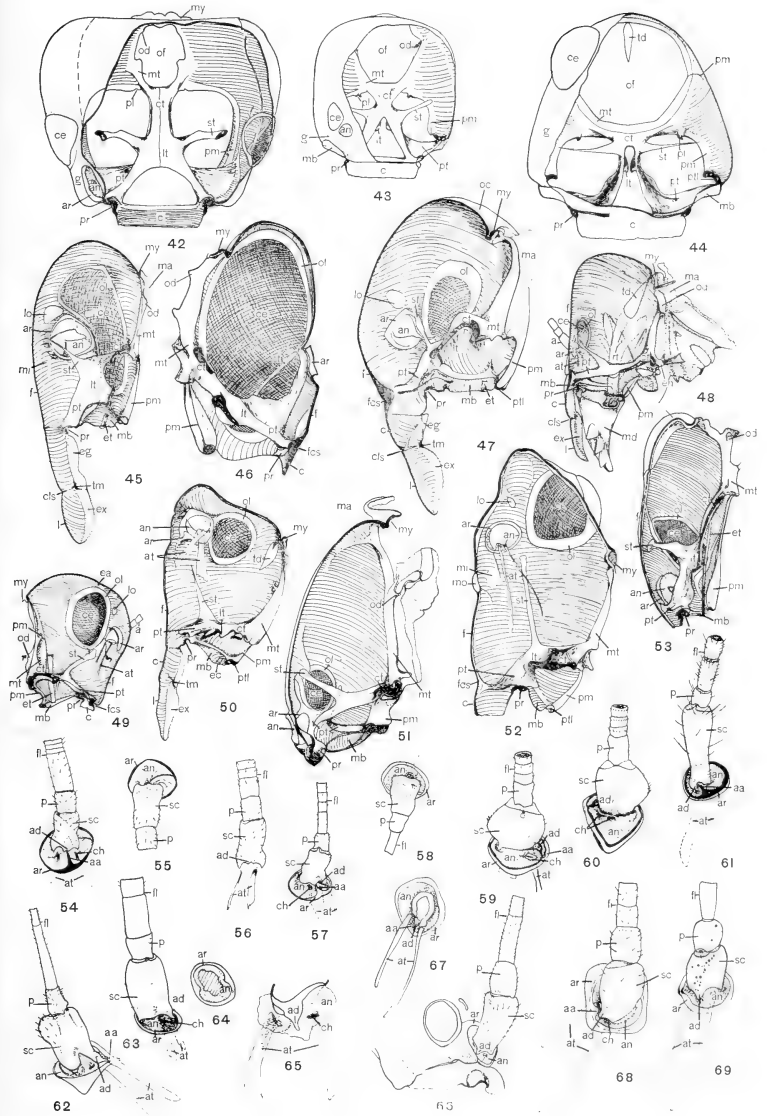
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PLATE 4

EXPLANATION OF FIGURES

70 to 92 Mandibles with tendons.
70, 80, 85, 86, 90, 91 Dorsal aspect of sinistral mandible.
71, 81, 82, 87, 92 Ventral aspect of sinistral mandible.
73, 76, 78, 79, 84, 89 Dorsal aspect of dextral mandible.
72, 75, 77, 83, 88 Ventral aspect of dextral mandible.
70 to 73 Blatta orientalis.
74 to 76 Mantis religiosa. 74, mesal aspect of dextral mandible.
77 and 78 Gryllus pennsylvanicus.
79 and 82 Orchelimum vulgare.
80 and 81 Diapheromera femorata.
83 to 85 Stenopelmatus sp.
86 and 87 Anisolabis maritima.
88 to 90 Tettix arenosus.
91 and 92 Melanoplus differentialis.
93 Anisolabis maritima, dorsal aspect of sinistral maxilla.
94 Anisolabis maritima, ventral aspect of sinistral maxilla.
95 Anisolabis maritima, caudal aspect of cardo.

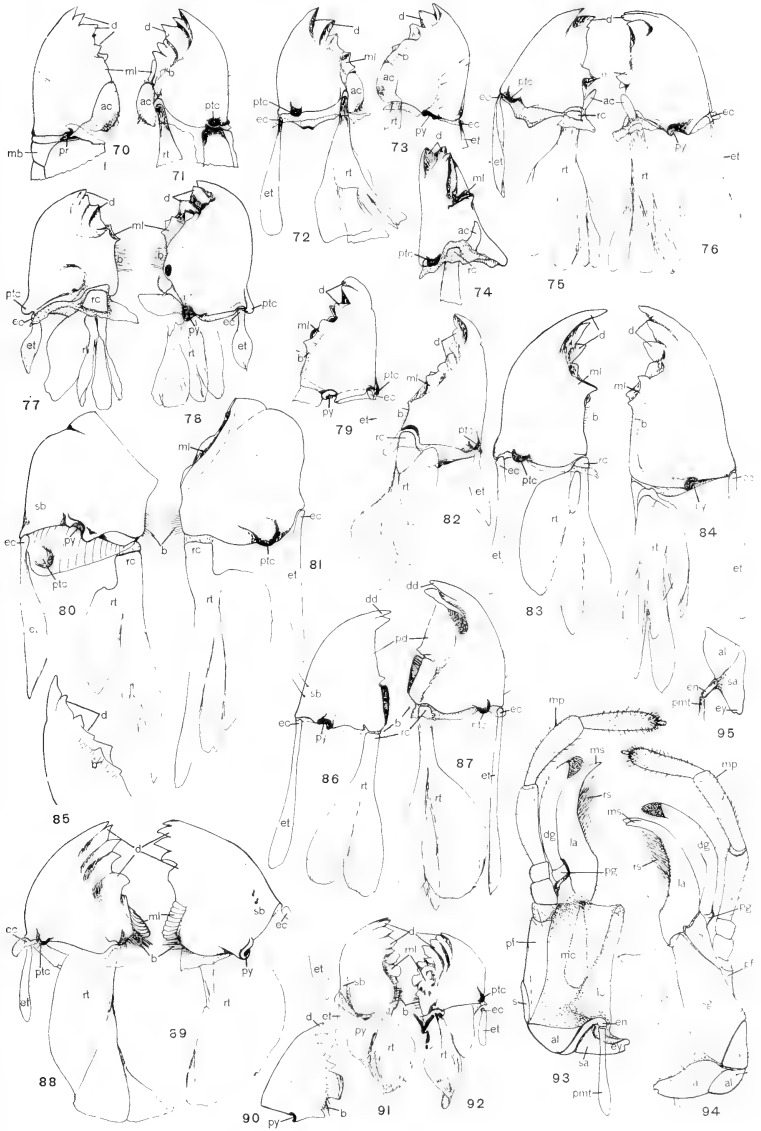
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PLATE 5

EXPLANATION OF FIGURES

96 to 119 Maxillae.
98, 99, 101, 106, 108, 113, 115, 117 Caudal aspect of cardo.
96, 97, 103, 107, 109, 114, 116, 118 Ventral aspect.
100, 102, 104, 105, 110, 111, 112, 119 Dorsal aspect.
96, 104, 106 Mantis religiosa.
97, 98, 100 Blatta orientalis.
99, 102, 103 Gryllus pennsylvanicus.
101, 109, 111 Stenopelmatus sp.
105, 107, 108 Orchelimum vulgare.
110, 115, 116 Diapheromera femorata.
112 to 114 Melanplus differentialis.
117 to 119 Tettix arenosus.
99[1] Tettix arenosus, mesal aspect of the distal portion of lacinia, showing the arrangement of maxadentes.

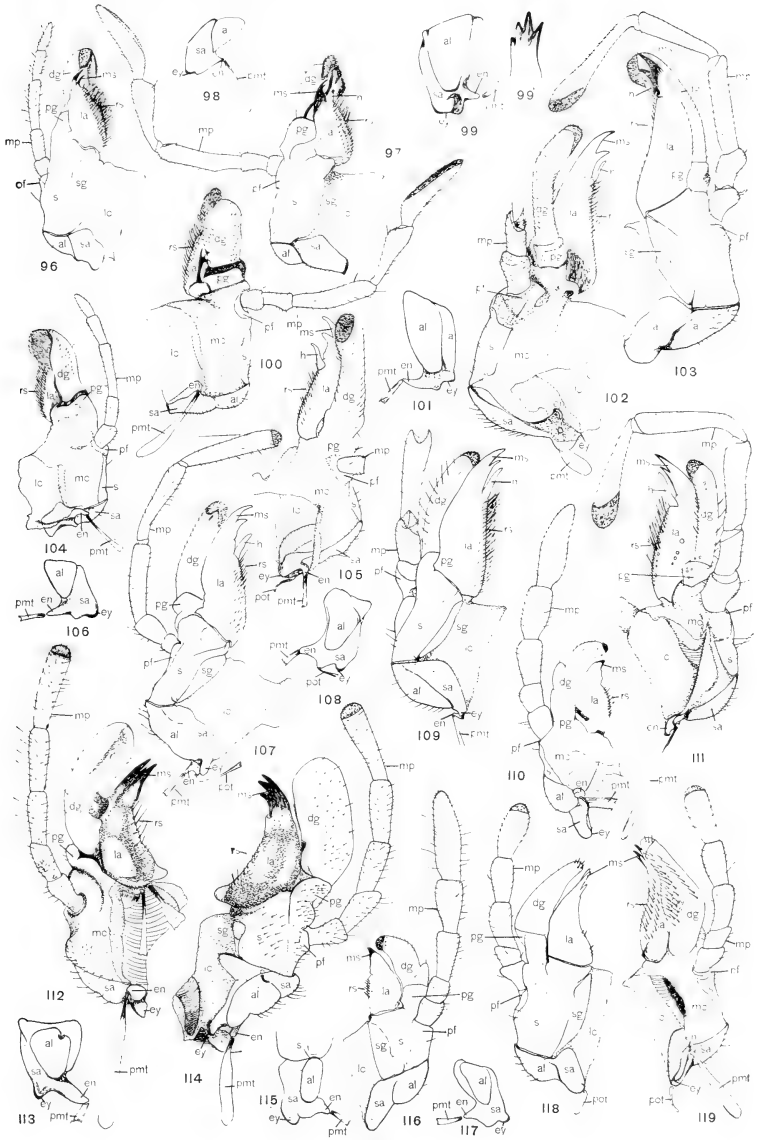
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PLATE 6

EXPLANATION OF FIGURES

120 to 135 Labium.
120 to 127 Ventral aspect.
128 to 135 Dorsal aspect with hypopharynx turned over to show its ventral aspect.
120, 128 Blatta orientalis.
121, 129 Mantis religiosa.
122, 130 Gryllus pennsylvanicus.
123, 131 Stenopelmatus sp.
124, 135 Melanoplus differentialis.
125, 133 Orchelimum vulgare.
126, 132 Tettix arenosus.
127, 134 Diapheromera femorata.

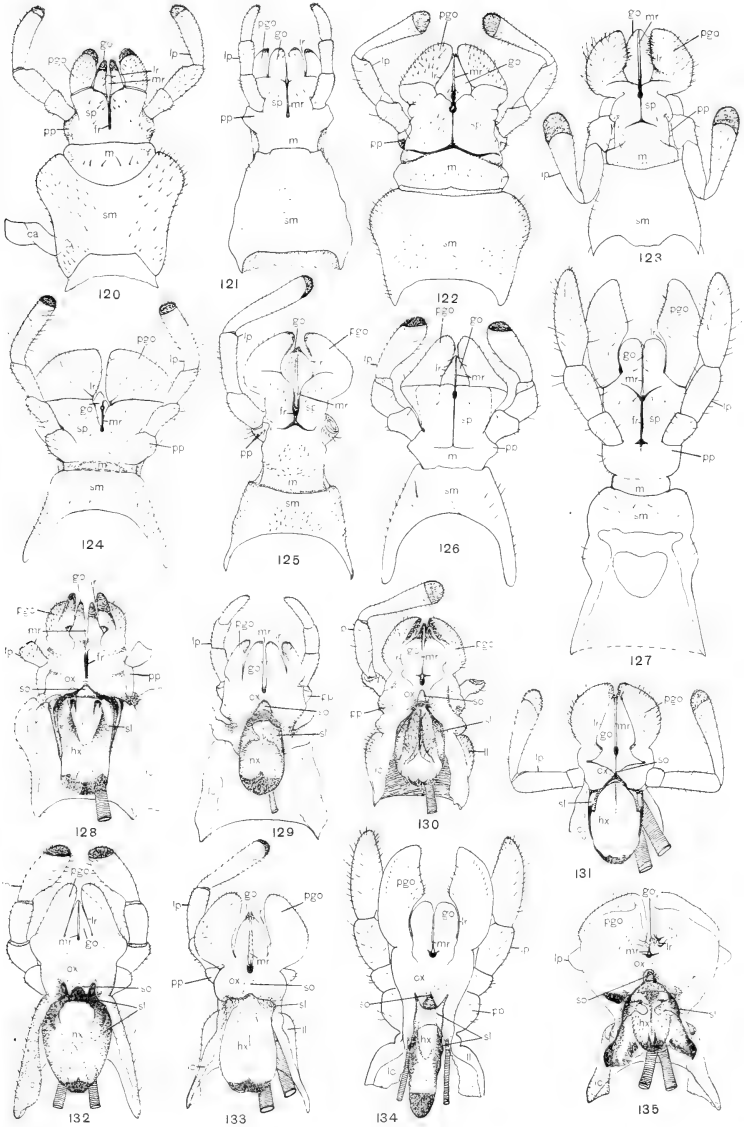
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PLATE 7

EXPLANATION OF FIGURES

136 and 137 Anisolabis maritima, labium. 136, ventral aspect; 137, dorsal aspect with the hypopharynx turned over to show its ventral aspect.

138 Anisolabis maritima, mouth-parts dissected out and prepharynx flattened out to show its parts.

139 Gryllus pennsylvanicus, sagittal section of head showing prepharynx.

140 Orchelimum vulgare, sagittal section of head showing prepharynx.

141 Mantis religiosa, mouth-parts dissected out showing lateral aspect of prepharynx.

142 Diapheromera femorata, sagittal section of head showing prepharynx.

143 Anisolabis maritima, sagittal section of head showing prepharynx.

144 Stenopelmatus sp., sagittal section of head showing prepharynx.

145 Tettix arenosus, mouth parts dissected out showing lateral aspect of prepharynx.

146 Anisolabis maritima, part of salivia near salivos showing the fusion to mesarima of labium.

147 Anisolabis maritima, lateral aspect of hypopharynx.

148 and 149 Gryllus pennsylvanicus, dorsal and lateral aspect of parapharynx.

150 Stenopelmatus sp., lateral aspect of parapharynx.

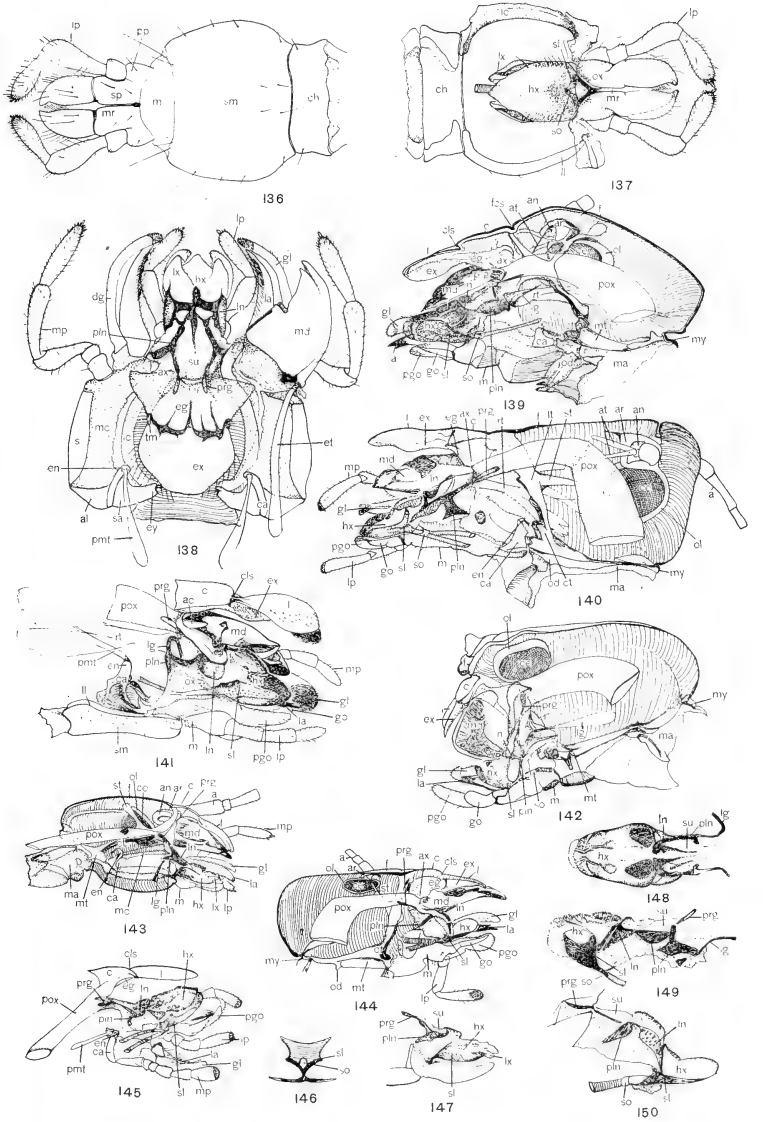
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PLATE 8

EXPLANATION OF FIGURES

151 Blatta orientalis, showing different structures of mouth-parts. This view is obtained by dissecting out the mouth-parts intact and arranging the prepharynx so that the observer looks straight down into the mouth cavity. In this way practically all the structures which belong to the mouth-parts can be shown in one drawing.

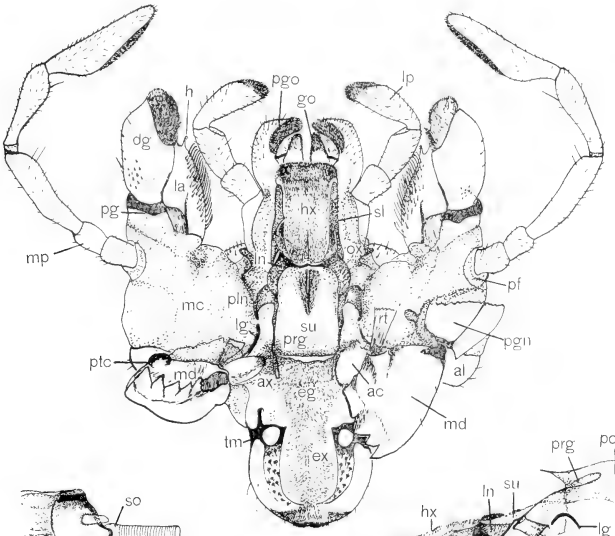
152 Blatta orientalis, ental aspect of the ventrocaudal part of hypopharynx showing the cuticular pouches, salivial bars and salivos.

153 Blatta orientalis, lateral aspect of parapharynx.

154 Blatta orientalis, sagittal section of head showing the lateral aspect of prepharynx.

155 Melanoplus differentialis, sagittal section of head showing the lateral aspect of prepharynx.

156 Melanoplus differentialis, lateral aspect of parapharynx.



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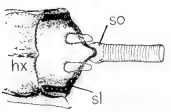

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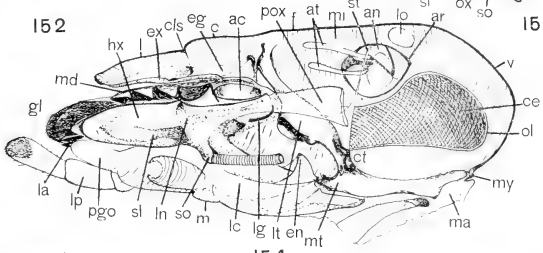

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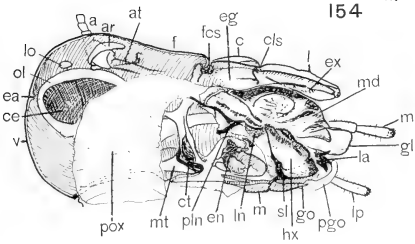

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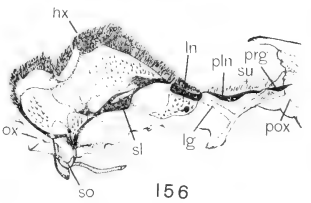

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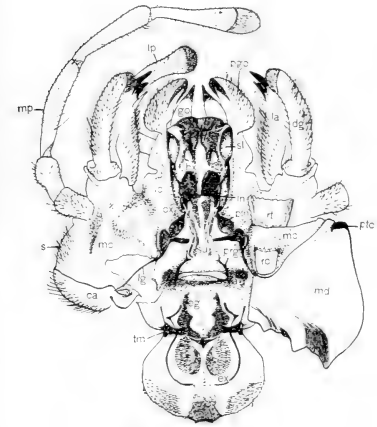
PLATE 9

EXPLANATION OF FIGURES

157 to 163 Mouth-parts, arranged in the same way as in figure 151.
157 Gryllus pennsylvanicus.
158 Orchelimum vulgare.
159 Stenopelmatus sp.
160 Tettix arenosus.
161 Mantis religiosa.
162 Melanoplus differentialis.
163 Diapheromera femorata.



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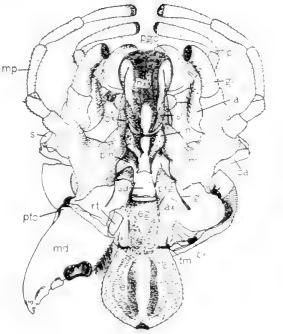

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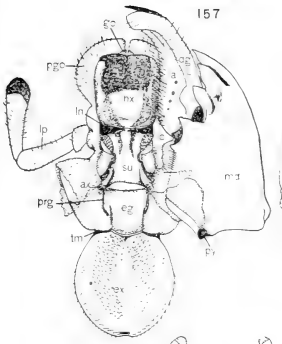

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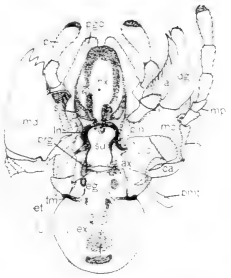

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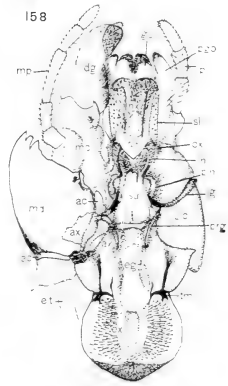

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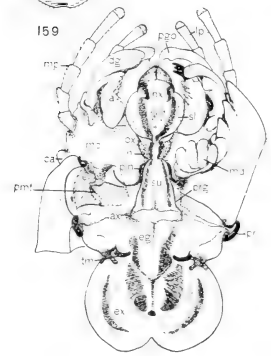

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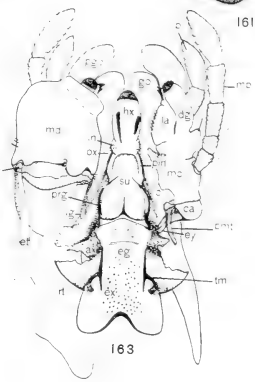

163

Resumen por los autores, William A. Kepner y Frank Helvestine.
Universidad de Virginia.

La faríngе de Microstoma caudatum.

1. La faríngе de este animal está provista de un disco chupador que le sirve para procurarse el alimento; la faríngе es mucho mas distensible que lo que se ha creido previamente. 2. A la función prehensil de la faríngе contribuye el ciego anterior del enteron. 3. Las secrecciones de las glándulas faríngeas paralizan a las Hydra. Esta parálisis es local, estando limitada a las partes del cuerpo de la presa que han sido ingeridas y no es permanente, recobrando la presa su actividad cuando es devuelta al exterior. 4. El epitelio que forra a la faríngе contiene núcleos esparcidos y es relativamente bajo. 5. La faríngе aparece después que se ha formado la parte fundamental del sistema nervioso central, estando completamente diferenciada antes que se separen los zooides de un Microstoma en vias de división. 6. De los órganos diferenciados, el sistema nervioso central mantiene la conexión mas íntima con los alimentos ingeridos por el padre, durante el desarrollo del nuevo zooide. 7. En el fondo de la faríngе en vias de desarrollo aparecen células que corresponden a las "Schliessenzellen" de las planarias; tales células forman la transición entre los epitelios faríngeo y entérico.

Translation by José F. Nonidez
Carnegie Institution of Washington

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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PHARYNX OF MICROSTOMA CAUDATUM

WM. A. KEPNER AND FRANK HELVESTINE, JR.

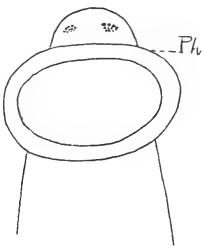
University of Virginia

ONE TEXT FIGURE AND THREE PLATES

Microstomum caudatum is frequently confused with Stenostoma leucops. The sexually immature individuals of these genera greatly resemble each other. The chief differences lie in their alimentary canals. The alimentary canal of Microstoma caudatum consists of two regions—the enteron and the stomodaeum. The elongated, sac-like enteron extends anteriorly over the dorsal side of the pharynx (fig. 1, *B*, and fig. 6, *A.S.*). This blind end or caecum of the enteron, lying over and projecting beyond the pharynx, constituted the chief diagnostic feature of Microstoma caudatum. The stomodaeum is represented by a short, glandular-walled tube—the pharynx (fig. 1, *A*). This is a simplex pharynx. It is rather more highly refractive than the other organs of the body and can, therefore, be readily recognized as the animal turns on its side or back. It is not definitely limited by a membrane, so that its glands stand out discrete projecting into the surrounding mesenchyme (fig. 1, *A*). When a sexually mature specimen lies on its back in a hanging drop, two openings of the body are conspicuous on the midline. One of these, the female genital pore, lies at the anterior limit of the posterior third of the body (fig. 1, *C*); the other, the mouth of the pharynx, lies at the posterior limit of the anterior fifth of the body (fig. 1, *M*). Von Graff ('09) describes and depicts the mouth as a longitudinal, slit-like opening but suggests no other details. Martin ('08) shows in his figure 10, plate 14, an open mouth, guarded by a circular disc-like lip. This is the nearest approach of any student to our observation of the details of this mouth. A living specimen, caught resting dorsal side down

in a hanging drop of water, will show that the mouth is provided with a flattened, circular lip which has many fine radiating lines upon or in it, thus presenting the appearance of a sucking disc (fig. 1, *D*).

Certain observations have been made upon the functions of the pharynx with its disc-like mouth. The pharynx functions in a twofold manner, a) as a prehensile and, b) as a secreting organ. Martin ('08, p. 268) says that when Microstoma attacks Hydra "it fixes itself for a short time by its posterior end in the neighborhood of the Hydra, and everts its pharynx to its full extent." He then refers to his figure 10 to illustrate a pharynx fully extended (text figure). This figure does not show adequately the



Text-figure Ventral aspect of anterior end of Microstoma to show mouth distended. From Martin ('08).

degree to which the pharynx of Microstoma may be distended. We starved a specimen for five days and then presented it with half of an oligochaete worm. The worm was at once attacked near its middle, the Microstoma's pharynx was distended in such fashion that the body assumed the contour of a laterally compressed funnel or cone. As the mouth of the rhabdocoele was thus distended, the prey, though wriggling much at its extremities, was sucked into the lumen of the enteron (fig. 2). After the prey had been drawn down to assume a U-shape, it was next handled in such manner as that one arm of the U was gliding from the mouth of Microstoma as the other was being dragged into it as indicated by arrows in figure 3. When the ingested

worm lay head-on within the enteron, ingestion of it was renewed and continued until completed, although the Microstoma was so gorged that the pharynx of the posterior zooid, not yet being in communication with the enteron, was everted (figs. 4 and 5). Thus it is seen that the mouth of the pharynx is distensible to a surprising degree and that the pharynx operates as a prehensile organ which can bring considerable force to play upon prey that is being ingested. The mechanism by which this forcible ingestion is accomplished is shown in figures 6 and 7. Figure 6 shows a sagittal section of an animal with its mouth nearly closed. The passage-way from pharynx to enteron is closed by a region of slightly peculiar cells. The anterior sac of the enteron is conspicuous and lies well up within the 'head' (fig. 6, *A.S.*). In forming a sucking apparatus or piston, the lips of the enteron are everted into the pharyngeal cavity. This eversion involves dragging the anterior sack of the enteron ventrally and posteriorly. The widened pharyngeal cavity now contains an everted knob of enteric tissue (fig. 7, *E.K.*), which can be drawn back into its resting position and thus cause a vacuum within the pharyngeal cavity. It is by the repeated everting and drawing back of this region of the alimentary canal, together with an alternate distending and contracting of the pharynx, that objects can be quite forcibly crowded into the enteron of Microstoma.

This prehensile function of the pharynx is supplemented by the work of the glands. Martin ('08) kept some Hydras in a solution of neutralroth, and "the vacuoles of the ectoderm of the hydras, which had been stained a pink color by the neutralroth, took a yellowish-brown color under the action of the digestive fluids, indicating that the secretion (of Microstoma) was of an alkaline nature, and possibly allied to trypsine." Martin said that under the conditions of ingestion the "tentacles of Hydra do not grasp the Microstoma, but remain extended almost parallel with its body, and it would appear as though the pharyngeal secretion has a paralyzing action on the Hydra." He further says that "in many cases, after a time, the Microstoma leaves its prey, and in such a case the Hydra does not seem much the worse for the attack" (p. 268).

That Microstoma can come up and tear off a tentacle of Hydra without the other tentacles or any other parts of the victim's body being disturbed has been frequently observed in this laboratory. When the entire Hydra is being ingested, the body of the prey contracts to a form shown by contour A, figure 8. Sometimes a Hydra that has been thus ingested is egested. Such a rejected polyp remains quiet, neither expanding nor further contracting, as if both its superficial or longitudinal muscles and its deeper or circular muscles had been paralyzed. This condition of complete paralysis is passed within an hour. Within that period the recovery is complete. An interesting observation in one case showed that the longitudinal muscles were not completely paralyzed. For in this case, throughout the process of ingestion the Hydra has a shape approximately like that indicated by contour A, figure 8. As soon, however, as the polyp was crowded into the enteron by the side of an oligochaete, that had been previously ingested by the Microstoma, it became spherical. Here, then, the longitudinal muscles had the power to contract further when they were more severely stimulated by the secretions of the enteron. The partial paralysis of an animal ingested is local and confined to the part of the body which has been taken into the pharynx. That this is the case is indicated by a second observation. A Microstoma was ingesting a Hydra from its aboral end. The polyp, during the process, had a shape indicated by contour A, figure 8. The rhabdocoele gave up and left its prey after having held the aboral half of the Hydra in its pharynx for nearly a half-hour. The Hydra, after being egested, showed the power to move and to elongate only the oral half of its body. For nearly an hour the oral end would elongate, ply to and fro, contract and expand again; but through all of this activity the aboral end that had been ingested remained quiet, so that the Hydra, when its oral half was distended, had the form indicated by contour B in figure 8. Here it is apparent that the paralyzing effect of Microstoma had influenced the deeper or circular muscles of Hydra's body. Thus it is indicated that both the outer (longitudinal) and the inner (circular) muscles of Hydra are paralyzed by an attack from Microstoma, if it be sustained long

enough. This paralysis, however, is confined only to the regions of the body taken into the pharynx, and is not complete, for within an hour the animal had fully recovered.

The observations last cited indicated a striking difference of reaction on the part of the Hydra, depending upon the region of the polyp which was attacked by Microstoma. We have frequently seen Microstoma snip away a tentacle without disturbing other regions of the body than the tentacle involved. However, when the attack is made upon the aboral end of a Hydra (lying free—not fixed), the response of the polyp is general—the entire polyp (body and tentacles) contracting. This suggests an interesting line of speculation. Parker ('17 a) found that the tentacles of actinians were but 'slightly sensitive,' while the aboral or pedal regions were highly sensitive to mechanical or contact stimuli. On the other hand, the tentacles of these polyps were very sensitive to chemical stimuli (juice of mussels) while the aboral or pedal regions were but slightly sensitive to such stimulation. In the light of Parker's observations, it is suggested that there may be in Hydra, as in actinians, paths of 'specialized transmission.' If it be that the tentacles of Hydra are more sensitive to chemical stimuli and the aboral end is more sensitive to mechanical stimuli, then our observations indicate that the preliminary phase of Microstoma's attack upon Hydra is mechanical, the chemical phase ensuing only after the prey lies within the pharynx and enteron.

The histology of the pharynx indicates that it is prepared to function in a chemical manner, for it is highly glandular. The pharynx, in its resting condition, is spindle shaped, with a lumen in the form of an oblate spheroid. The lumen is lined with a strongly ciliated, sparsely nucleated epithelium of low cells (fig. 9, *Ep*). This epithelium is frequently interrupted by the ducts of the many unicellular glands which radiate into the mesenchyme from all sides of the pharyngeal wall. There are many indefinite layers of these gland-cells. Those nearest the lining epithelium are the smallest, while those lying at the periphery of the pharynx are the oldest and largest (fig. 9, *A*, *B*, *C*). The smallest gland cells show no sign of secretion formation, having neither

conspicuous vacuoles nor secretion products within the cytoplasm (fig. 9, *A*). In the older cells vacuoles appear within the cytoplasm, become larger and more crowded with secretion products, until they occupy the greater part of their respective cells (fig. 9, *B*). The cytoplasm of such cells is conspicuous only at the fundus of the cell body. In this basal mass of cytoplasm is the nucleus (fig. 9, *B*). It appears that only these large cells at the periphery discharge their contents into the lumen of the pharynx, for no empty vacuoles are found in the cells of intermediate size. Many empty large cells occur at the periphery of the pharyngeal wall (fig. 9, *C*). The staining reaction of the empty cells is wholly basic, taking the acid dyes. Bordeaux red, for example, or eosin stains all the details of these empty cells red. This is in marked contrast to the reaction of the young and secreting cells. In these the nuclei are highly acid in their reaction, while the secretion granules show a great affinity for the basic dyes.

The origin of the pharynx is readily studied in dividing specimens. Animals are not infrequently found which show two planes of division, thus presenting a chain of three zooids, while specimens with two zooids are very common at any season of the year.

The division of a Microstoma to form two zooids involves the division of the enteron, the formation of a new 'brain,' new ciliated pits, gonads, and pharynx. We are concerned here only with the origin of the pharynx; but it is an interesting fact that the developing 'brain' of the posterior zooid remains in contact along its anterior dorsal surface with the enteron until the pharynx has become well established and even connected with the enteron (figs. 10, 11, and 12, *Br.*). This 'brain' makes its appearance earlier than does the anlage from which the pharynx is differentiated. The pharynx arises as an invaginated sac of ectodermal epithelium. At first the wall of the young pharynx is composed of but a simple, ciliated columnar epithelium without any glandular differentiation (fig. 11). Soon, however, a threefold differentiation of the cells of the ectodermal sac sets in. This differentiation involves, a) a lining epithelium of the pharynx;

b) the gland cells which leave the epithelium to sink radially into the mesenchyme, and, (c) a few cells at the fundus of the young pharynx. These last cells appear to be modified as transitional cells between the epithelium lining the pharynx and the enteric epithelium. We may therefore call them transitional cells (figs. 11 and 12, *T.C.*). These transitional cells increase in number and form a conspicuous plug protruding into the lumen of the pharynx at the time communication between pharynx and enteron is established. Both in position and histological detail these cells suggest the 'Schliesszellen' which Mattiesen ('04) describes for the developing pharynx of a planarian embryo. The region of the transitional cells never becomes extensive. The most extensive mass of cells in the pharynx is that of the gland cells. Here the differentiation is rapid and very many gland cells are formed before the young pharynx communicates with the lumen of the enteron. Indeed, many of the gland cells of an advanced pharynx are filled with secretion granules before the lumina of pharynx and enteron are continuous. No empty gland cells, however, have been found in a pharynx that does not lead into the enteron, though the fission of the animal had advanced to where the parent enteron had completely divided. The posterior zooid is thus provided with a pharynx whose glands are charged with secretion products, ciliated pits and a 'brain' which lies quite near the cleavage plane. The new zooid is completed with the formation of a rounded anterior extremity.

SUMMARY

1. The pharynx is provided with a sucking disc which serves the animal in securing food. The pharynx is much more highly distensible than previously described.

2. The pharynx is aided in its prehensile functioning by the anterior caecum of the enteron.

3. The secretions of the glands of the pharynx paralyze Hydras. This paralysis is local, confined to the parts of the prey's body that have been ingested and is not permanent, the prey recovering if egested.

4. The lining epithelium of the pharynx is sparsely nucleated and relatively low.

5. The pharynx arises after the fundament of the central nervous system has made its appearance. The pharynx is fully differentiated before the zooids of a dividing Microstoma separate.

6. Of the differentiating organs the central nervous system maintains the most intimate connection with the parent food supply throughout the development of the new zooid.

7. Cells corresponding to 'Schliesszellen' of Planaria appear at the fundus of developing pharynx. These are transitional cells between the pharyngeal and enteric epithelia.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 Ventral aspect of sexually mature specimen. *A*, pharynx; *B*, anterior sack of enteron; *C*, opening of vagina; *D*, sucking disc; *M*, mouth. × 75.

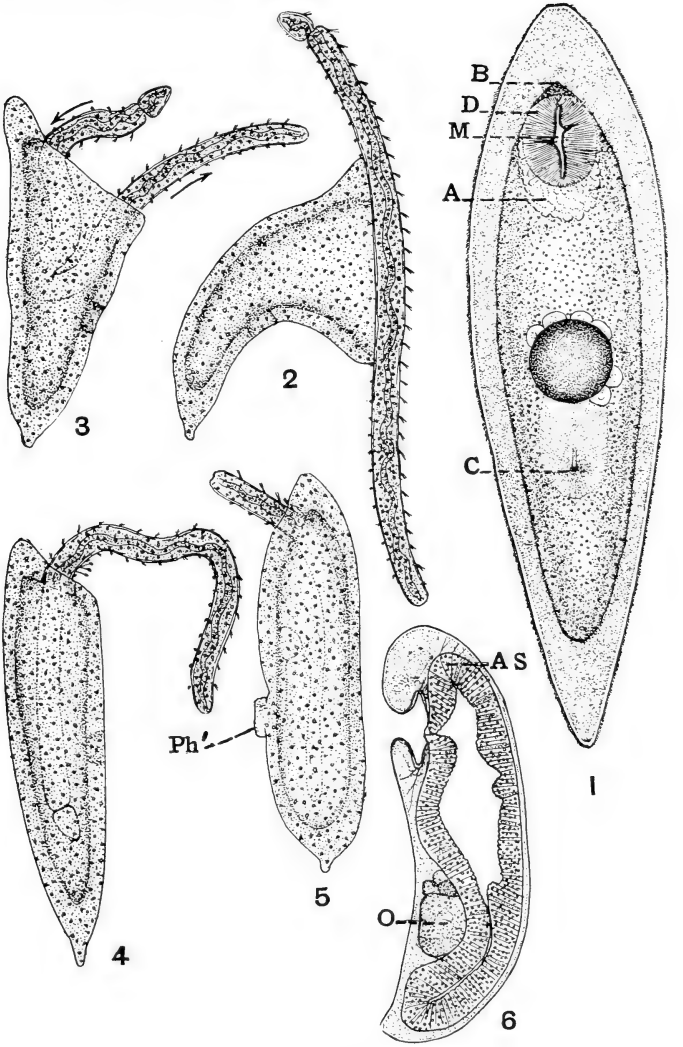
2 Specimen loaded with nematocysts from Hydra attacking a living half of an oligochaete. × 50.

3 Oligochaete, partly ingested, being handled in such manner as that one end is moving out of mouth of Microstoma, while the other end is being dragged into mouth as shown by arrows. × 50.

4 Prey lying end on and half ingested. × 50.

5 Prey almost ingested. The Microstoma so badly gorged that the pharynx of posterior zooid (*Ph'*) is everted. × 50.

6 Sagittal section of specimen with closed mouth. Anterior sac of enteron conspicuous (*A.S.*). *O*, oocyte. × 75.


B
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Ph'
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PLATE 2

EXPLANATION OF FIGURES

7 Sagittal section of specimen with mouth open. The enteric epithelium of anterior sac and of floor of 'stomach' is everted at *E.K.* to form a sort of piston within the lumen of partly distended pharynx. At fixation specimen ruptured along fission plane (*F*). *O* and *O'*, oocytes of two zooids. × 75.

8 Hydra that had been half ingested from aboral end. During ingestion polyp had contour A. When egested Hydra could distend and move oral half, but aboral half of body was paralyzed. The polyp, with paralyzed aboral half, had contour B. × 50.

9 Part of pharyngeal epithelium (*Ep*) with gland-cells. *A*, young gland-cell; *B*, gland-cell at maximum secretion phase; *C*, emply gland-cell; *D*, ducts of gland-cells passing through lining epithelium of pharynx. × 1500.

10 Frontal section of two zooids. Parent enteron completely divided, but 'brain' of posterior zooid yet lies in intimate contact with the enteron of anterior zooid. *Br*, 'brain' of posterior zooid; *E*, enteron of anterior zooid. × about 100.

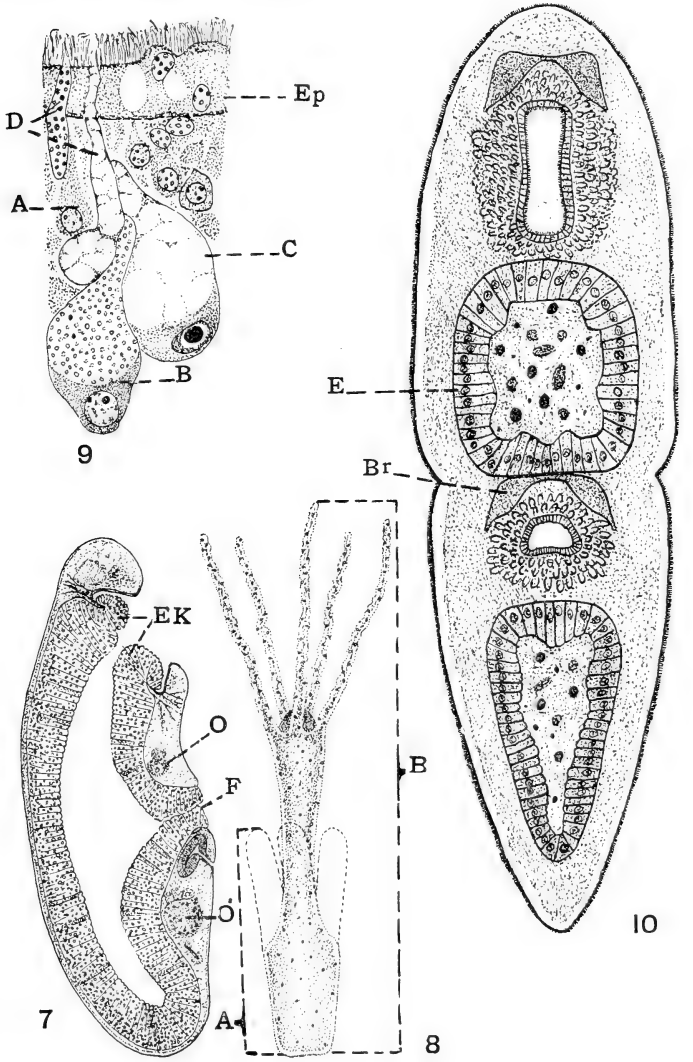
Ep
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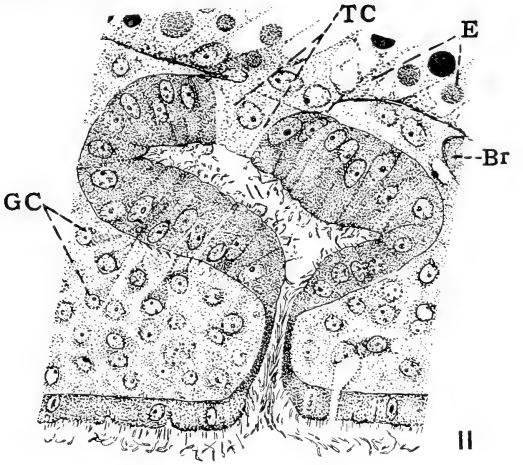
PLATE 3

EXPLANATION OF FIGURES

11 Section of a young pharynx showing beginning of differentiation of gland-cells, *G.C.*, and a transition cell differentiated, *T.C.* *Br*, 'brain;' *E*, enteric epithelium. × 1400.

12 Section of an older pharynx than one shown in 11. Two transitional cells shown, *T.C.* Many gland-cells present, *G.C.* *Br*, 'brain;' *E*, enteric epithelium. × 1400.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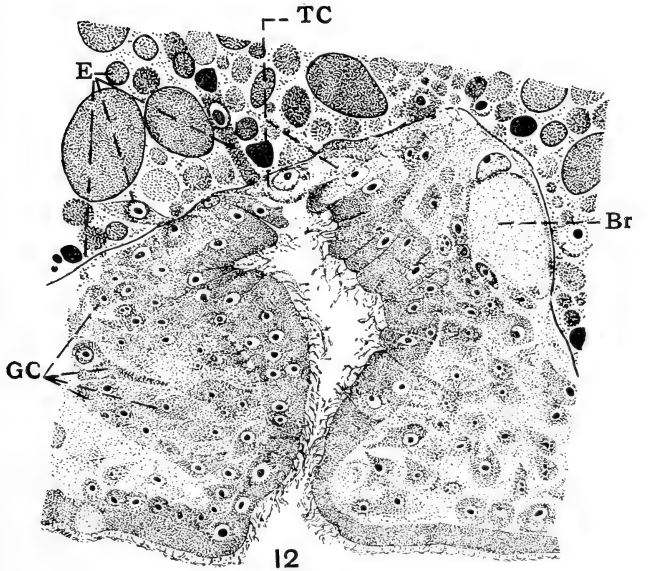
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Resumen por el autor, H. D. Reed.
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La morfología del aparato transmisor del sonido en los anfibios caudados y su significación filogenética.

En los anfibios dotados de cola existen dos tipos morfológicos de aparato transmisor del sonido. En el tipo mas generalizado, la columela y el opérculo son distintos, en lo referente a la fusión de una con el otro. En el otro tipo los representantes de la columela y el opérculo están fusionados entre sí, formando de este modo una placa fenestrada. Esta particularidad hace posible la división del conjunto del órden en dos legiones: una que incluye a los Amblystomidae, Cryptobranchidae, Salamandra, Triton y Diemictylus, y los Sirenidae; la otra que comprende a los Necturidae, Amphiumidae, Typhlomolgidae, Plethodontidae y Desmognathidae. El aparato definitivo generalizado es útil solamente en el medio ambiente terrestre y debe haberse originado en conexión con este medio. Las formas que actualmente son acuáticas han adquirido secundariamente este modo de vida. Otras tienden al modo de vida acuático, mientras que algunas han retenido las costumbres terrestres mas primitivas con la correspondiente estructura y modo de desarrollo.

Translation by José F. Nonidez
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THE MORPHOLOGY OF THE SOUND-TRANSMITTING APPARATUS IN CAUDATE AMPHIBIA AND ITS PHYLOGENETIC SIGNIFICANCE

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EIGHTEEN TEXT FIGURES AND SIX PLATES

INTRODUCTION

In earlier papers Kingsbury and Reed ('08 and '09) pointed out the existence of two structures composing the sound-transmitting apparatus of the tailed amphibians. One of these structures they called columella which is extraotic in origin and is connected, either directly or by a ligament, with the suspensorium of the lower jaw. In the typical state it functions as the organ of sound (jar) transmission during larval life. This condition is probably the one which prevailed in primitive Caudata. The other element was designated operculum which functions during adult (terrestrial) life. It makes its appearance at the period of transformation in such forms as Amblystoma and, upon completion, becomes connected with the suprascapula through the M. opercularis. The operculum arises as a circular plate of cartilage 'cut out' from the walls of the ear capsule caudad of the primary fenestra which is occupied by the columella and is, therefore, otic in origin. The secondary fenestra which it occupies is formed at the same time and by the same method as the element itself. As the operculum completes the formative period and assumes a functional rôle, the columella gradually fuses with the cephalic margin of the fenestra and probably ceases to function, at least, to any extent.

In these papers the authors discussed the various morphological relations of the columella and operculum and pointed out the evidence which seemed to favor the hyomandibular homology of

the columella. Comparisons were also made with the sound-transmitting apparatus of the Salientia.

There remain to be considered certain points that are concerned with the morphology of the fenestral elements within the various groups of the tailed amphibia themselves and the zoological significance of these structures. The Amblystomidae and Salamandra possess the two elements mentioned above. This is the most generalized state found among the terrestrial species. All other salamanders in the adult state possess only a single element filling the foramen vestibuli. Such forms are: Cryptobranchidae, Proteidae, Plethodontidae, Desmognathidae, Amphiumidae, Sirenidae, and Triton and Diemictylus. These groups include the majority of all living species. In certain of these forms the morphology of the single fenestral plate was demonstrated in the papers already mentioned. In Triton and Diemictylus the single element possesses only the characteristics of the amblystomid operculum; that is, it is without stylus and suspensorial connections, but possesses a perilymphatic prominence, between which and the supraclavicle the M. opercularis extends. Development showed that the single element in Diemictylus and Triton is the operculum or that which corresponds to the caudal of the two elements in the Amblystomidae and Salamandra. It further showed that the columella is present, but disappears as such at an early period through complete fusion with the cephalic lips of the fenestra (fig. 21). In a similar manner it was revealed that the single element of the Cryptobranchidae represents the columella of the amblystomid forms, it being wholly of extraotic origin and possessing only the suspensorial connections.

With regard to the other forms, however, definite information was not forthcoming at that time to account for the presence of a single element in the fenestra. In the Plethodontidae and Desmognathidae, for example, the single fenestral element possesses at one and the same time the connections and relations of both columella and operculum (fig. 22). Through a stylus and ligament the cephalic end of the plate is connected with the suspensorium, and in its caudal portion there is a well-defined peri-

lymphatic prominence which is placed in connection with the supraclavicle by the M. opercularis. More recently, the present writer (Reed, '15) has interpreted certain aspects of the problem bearing upon the morphology of the single fenestral plate in Necturus and has pointed out that in its nature it differs from the single element in both the Triton-Diemictylus group and the Cryptobranchidae, although apparently agreeing with the latter in skeletal connections, form, and general estate. A brief review of these results will appear later in the present paper.

For the present study there were available series of both larvae and adults of most species considered, but some series were found lacking in important stages. Of those which were chosen to serve as type studies for a given taxonomic group an attempt was made to obtain developmental stages as numerous and as close together as possible. The results follow: Spelerpes bislineatus, larvae 10, 15, 17, 19, 20, 21, 23, 25, 28, 31, 34, 37, 43, and 55 mm., respectively; Desmognathus fusca, larvae of 10, 26, 27, and 30 mm.; Amphiuma means, embryos of 30 and 33 mm., a mature larva and a newly transformed adult; Necturus embryos of 11, 12, 15, 16, 17, 18, 19, and 20 mm. and larvae of 21, 22, 23, 24, 25, 26, 35, 40, 43, 44, 48, and 70 mm. in length.

THE PLETHODONTIDAE

Spelerpes bislineatus was chosen as the type study for the family Plethodontidae, chiefly because of the relative ease of procuring material and partly because it was considered a generalized member of the family. As is usual in the Plethodontidae, the ear capsule is fully ossified in the adult except the lips of the fenestra and those points where connection is made with the processes of the palatoquadrate. Both the fenestra and the fenestral plate are relatively elongate, but narrow in the vertical diameter, as shown in figure 26. The stylus, a slender rod, is attached to the cephalic portion of the plate and extends forward and slightly upward where it joins the ventral edge of the squamosum. In adults as large as 57 mm. there is no connection of stylus and os quadratum unless a very slender and

doubtful strand of mesenchymal tissue extending between the two is to be so considered. The ossification of the fenestral plate is characteristic of the whole family Plethodontidae. The central portion is completely ossified while in the periphery cartilage persists between the outer and inner plates of bone. This is quite in contrast to the amblystomid type where an inner and outer shell of bone enclose cartilage at all levels. The central portion of the plate is relatively thin, and ossification begins here. The reason for the thinness of the plate at its center and its early and complete ossification is to be found in certain developmental conditions with a morphological significance, as well as the tendency in these forms to a reduction of the chondrocranium beyond that found in the Amblystomidae. This point will be discussed later.

One of the characteristic features of the fenestral plate of Spelerpes bislineatus is in the relation of the stylus to the plate itself. As stated above, the stylus joins the plate in its cephalic portion, but, even in the adult, the fusion is not so complete that the identity of the former is lost. In young adults the entire stylus is composed of a shell of bone surrounding a few cartilage cells. In its caudal extremity, where it joins the fenestral plate, the bony shell increases in thickness with a resulting diminution of cartilage within. The stylus, after first touching upon the plate, extends caudad a very short distance, in some cases less than 1 mm., but it is not completely incorporated with the plate substance. The cylindrical sheath of bone can be made out distinctly at all levels, although an anchylosis of the two structures takes place (fig. 1). At a level of 50 μ caudad of that of figure 1, the stylus disappears and only here does there seem to be any fusion between the two elements. The significance of this relation is revealed only through development. The connection of the definitive fenestral plate with the ear capsule is in its ventrocephalic margin where the cartilage of the two structures is continuous (fig. 22). This connection at all stages is relatively narrow, and in this, as well as position, it differs from any otic connection of fenestral elements in the amblystomid forms where the connection is more extensive and

represents a secondary relation. From the morphological viewpoint this connection is one of the most important structures of the whole sound-transmitting apparatus in the Plethodontidae, Desmognathidae, and others. For this reason and for the sake of easy reference later, it will be termed the isthmus fenestralis.

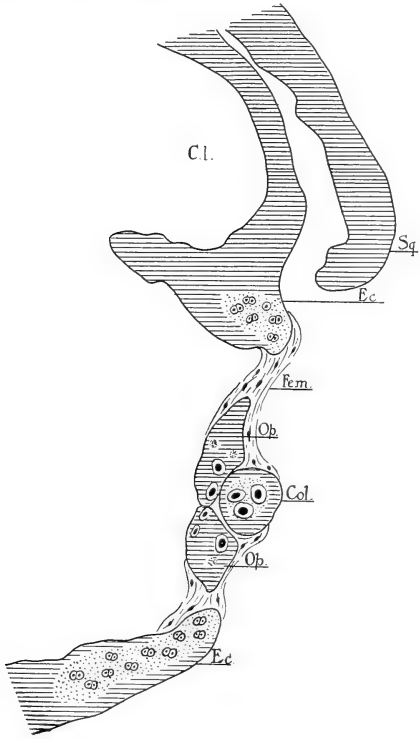


Fig. 1 Transection through the fenestra vestibuli of an adult Spelerpes bislineatus at the level of the stylus columellae. *C.l.*, canalis lateralis; *Col.*, stylus columellae (columella); *Ec.*, ear capsule; *Fe.m.*, fenestral membrane; *Op.*, fenestral plate representing the operculum; *Sq.*, squamosum.

In the caudal portion of the plate is a perilymphatic prominence, in a depression of which the M. opercularis is attached (fig. 22).

From the preceding paragraph it is obvious that Spelerpes and other genera have a structure which combines the characteristics of both columella and operculum of the amblystomid type. The natural inference is that in these forms the single fenestral element represents some sort of fusion of columella and operculum. With limitations, this statement is true, but the significance of 'fusion' in this relevancy becomes evident only after the examination of numerous developmental stages. Structures which are here considered to be the homologues of the columella and operculum are present, as was mentioned briefly in an earlier communication (Reed, '14) and in the nature and order of appearance of these structures in Spelerpes there is identity with Amblystoma; that is, the columellar representative appears first, followed by an element of otic derivation which may be compared with the operculum.

The proton of the columella appears first as a cord of cells extending from the squamosum to the region of the fenestra, while the plate filling this opening appears later. The development of these structures in Spelerpes is much retarded as compared with Amblystoma. In the latter, embryos 4 mm. long possess a well-defined columellar cord in its typical position and relations, while in Spelerpes bislineatus embryos 8 mm. long this whole region is filled with undifferentiated mesenchyme. In embryos 10 mm. in length the columella is discernible as a dense cord which bears a significant relation to the hyomandibular cleft which is seen as a very pronounced continuation of the oral epithelium reaching beyond the ventral border of the ear capsule (fig. 2), but the double folds do not separate to form a cavity. Just above the dorsal end of this cleft the proton of the columella may be seen as a dense mass of cells which, farther caudad, extends ventrad toward the fenestra, where it ends abruptly. In all of its relations it occupies the typical position above the facial nerve and between the artery and vein of this region. The columella at this stage is a dense segregation of

cells which comes into close relation with the caudal side of the hyomandibular cleft, a relation which is not lost until late larval life. The ear capsule is clearly indicated by distinct and closely

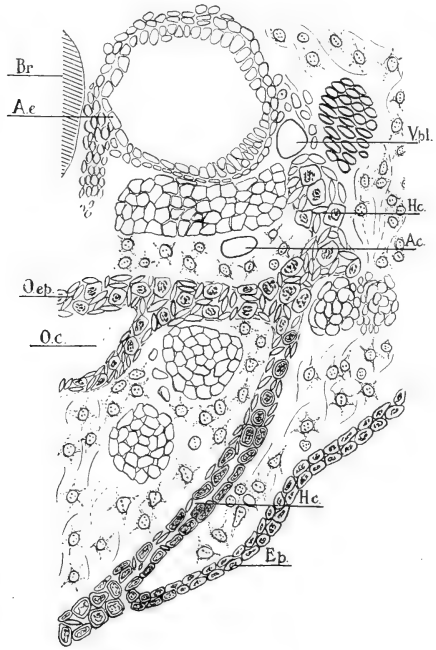


Fig. 2 Transection of the fenestral region of an embryo Spelerpes bislineatus 10 mm. long. *A.c.*, arteria carotis interna; *A.e.*, auditory epithelium; *Br.*, brain; *Ep.*, epidermis; *H.c.*, hyomandibular cleft; *O.c.*, oral cavity; *O.ep.*, oral epithelium; *V.p.l.*, vena petrosolateralis.

associated cells and the facial nerve and its branches may be distinctly traced.

In larvae which have reached a length of 15 mm. the columellar cord of cells extends caudad to the cephalic part of the fenestral membrane, against which it rests, but to which it is not closely

connected. From embryos 10 mm. long to the time chondrification of the columellar rod begins, it undergoes a decided reduction in diameter. It, however, retains its connection with the vestige of the hyomandibular cleft through a sheet of fascia which spreads out fan-shaped for attachment to the lining of the oral cavity. The dense rod of undifferentiated cells, so apparent in embryos of 10 mm., gives rise to both the columella and the sheet of fascia just mentioned. The two are distinct only as regards the more segregated nature of the cells which form the columellar proton.

In specimens 17 mm. long only slight changes have taken place over those conditions which obtain in the 15-mm. stage. The columellar cord is slightly thicker and lies at times a little closer to the fenestral membrane. Nothing, by way of structure or relations at this period of development denotes any morphological difference between the sound-transmitting apparatus of Spelerpes and that of Amblystoma.

Larvae from 19 mm. to 23 mm. in length are important as showing the first step in the formation of the fenestral plate, although considered apart from older stages, they are without significance and indeed might prove misleading. The whole history of development and morphology of these structures is evident only after the consideration of a complete and carefully selected series of stages. In the 19-mm. stage the fenestral lips, in the ventrocephalic extent, through proliferation and growth of their own cells, extend out into the fenestral membrane in the form of a triangle, the apex reaching the level of the columellar cord of cells. This growth of cells becomes the isthmus fenestralis which, as mentioned above, serves as the connection between the ear capsule and the definitive fenestral plate, and, as will be pointed out presently, constitutes the proton of the plate itself (figs. 23 and 24).

From this stage to those 21 mm. long the only noticeable changes are in the continued growth of the cells of the isthmus into the membrane and the elaboration of a cartilaginous matrix. The columellar cord has not yet begun to chondrify and there is no indication of a fusion of the two elements. The posi-

tion, extent, and direction of growth of the isthmus should be noted, in comparison with the columellar fusion with the cephalic lips of the fenestra in Amblystoma. Figures 20 and 22 illustrate the differences. In Amblystoma the columella, which is formed outside the ear capsule, comes to lie against the fenestral membrane, grows toward and secondarily fuses with the entire cephalic margin of the fenestra. In this case the columella (fenestral plate) may be said to be the active element in the fusions and the connections looked upon as extraotic tissue. In Spelerpes a narrow region of fenestral lips grows into the fenestral membrane, forming the isthmus fenestralis, which differs from this fusion in Amblystoma in three respects: *a*) in extent and location; *b*) the fenestral lips represent the active elements, and, *c*) the tissues forming the connection are strictly otic in source. The two, then, are in no respect alike. Figure 3 represents a section through the apex of the isthmus. Caudad of this level it suddenly decreases in height to the normal level of the fenestral lips.

In larvae 23 mm. long chondrification has occurred in the columellar cord at the level of the apex of the isthmus. It rests close against the fenestral membrane, but is not included within its tissues. This represents the initial step in the formation of the stylus columellae, which later, through chondrification, extends to the edge of the squamosum. The isthmus, through further growth into the fenestral membrane, reaches the dorsal level of the stylus, and there are indications that a few cells at the growing edge are about to extend further into the membrane beyond the level of the stylus and independent of it. The matrix of the two elements comes into contact. An important observation in this respect is that the cells invading the fenestral membrane from the isthmus lie entad of the stylus, and it is due to the growth of the former that the final connection is established. Thus here, as in the formation of the isthmus, the otic tissue is to be considered the aggressive element. At this stage the columellar cord and that portion which has chondrified as stylus occupy those relations to the fenestral plate which are maintained throughout life. It never enters more exten-

sively in these relations, although increasing in size along with the growth of the animal. The further development of the fenestral plate is concerned with the growth and extension of the original isthmus into all parts of the fenestral membrane. Figures 23, 24, 25, and 26 show the early mode of invasion. When first formed, the dorsal growth of the isthmus is triangular, with the apex reaching dorsally toward the stylus columellae, entad of which it has a tendency to pass. In the 28-mm. larva this cartilage has extended along the chondrified stylus, both caudad and cephalad, until the triangle is reversed in position (fig. 24). During this same period it has invaded the membrane entad of the stylus, against which the latter lies, and in one specimen it reached a level dorsad of that of the stylus.

The plan of growth is still more clearly outlined in the 31-mm. stage where the fenestral plate has spread for a considerable distance into the membrane in the dorsal and caudal directions (fig. 25). When the stylus joins the plate the cartilage of the two, in the posterior extent of the stylus, comes in contact, and fusion takes place, though both may be distinguished by the size of the cells or by staining reactions or both. While new cartilage is being formed all about the free edge of the plate, growth is not uniform. There are two points, one above, the other below the stylus, where growth is most active. This results in two chondrified rod-like structures extending caudad into the membrane, as shown in figure 25. In this particular specimen, these rods had a tendency to enclose an unchondrified area of the fenestral membrane. In their caudal growth the rods have nothing to do with the stylus or with the cells proliferating from it. This condition is perhaps better illustrated by transections. The growth from the original invading isthmus itself is shown in figure 4 (*d.g.*) of a section taken at a level marked *s* in figure 25. It also shows the independence of the columella and fenestral membrane at this level. A section through the caudal half of the fenestra (fig. 5), at a level marked *c.s.* in figure 25, shows the curved lower bar of the developing plate composed of cartilage cells within the fenestral membrane, which contrasts with the location of the cells of the columella in

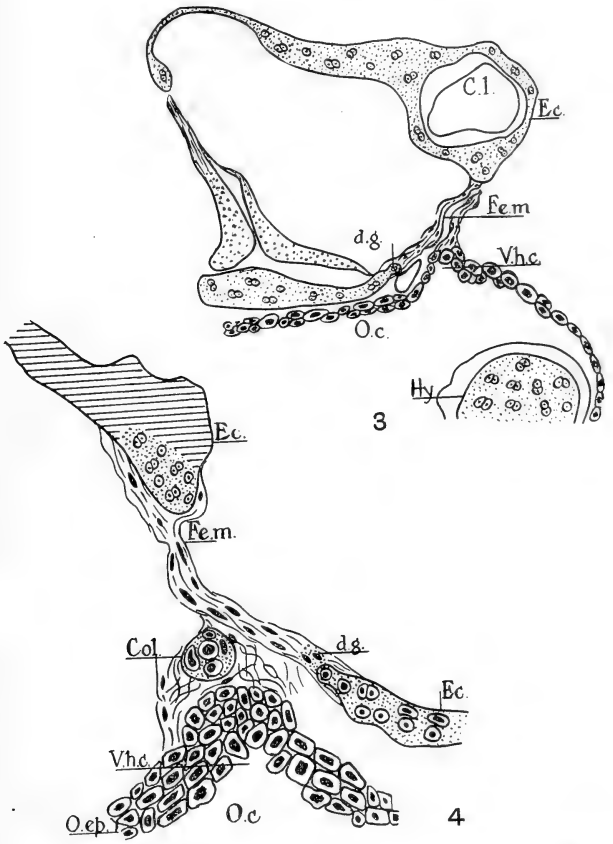
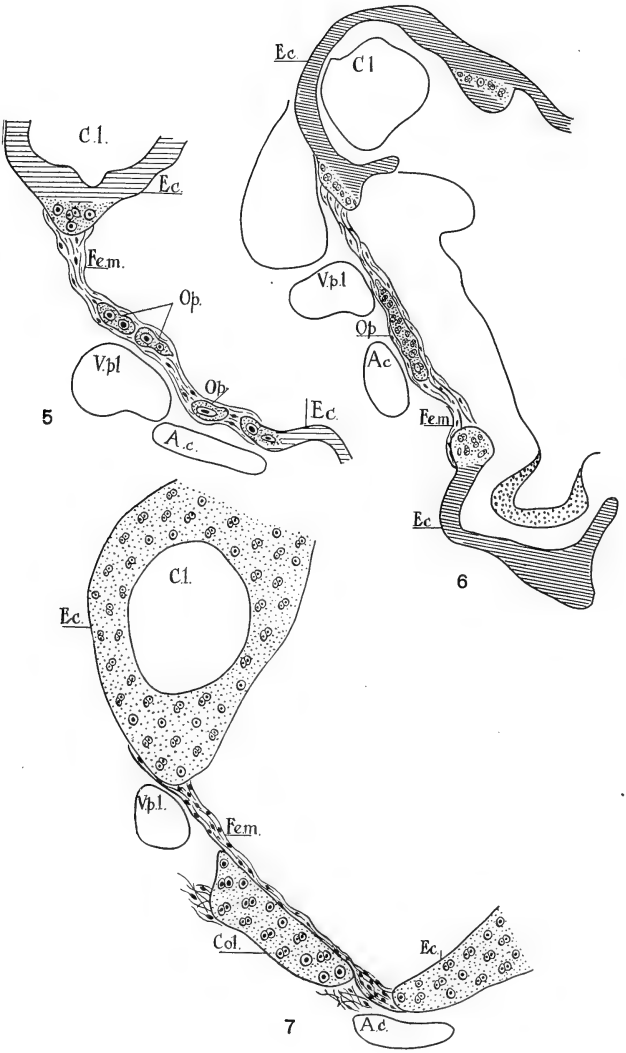


Fig. 3 Transection through the cephalic portion of the fenestra vestibuli of a larval Spelerpes bislineatus 20 mm. long. *C.l.*, canalis lateralis; *d.g.*, lips of the fenestra vestibuli growing dorsad into the fenestral membrane to form the isthmus fenestralis and to contribute to the formation of the fenestral plate; *Ec.*, ear capsule; *F.em.*, fenestral membrane; *Hy.*, hyoid; *O.c.*, oral cavity; *V.h.c.*, vestige of hyomandibular cleft.

Fig 4 Transection through the fenestra vestibuli of a larval Spelerpes bislineatus 31 mm. long. *Col.*, columellar stylus; *d.g.*, advancing edge of the isthmus fenestralis; *Ec.*, ear capsule; *Fe.m.*, fenestral membrane; *O.c.*, oral cavity; *O.ep.*, oral epithelium; *V.h.c.*, vestige of hyomandibular cleft.


C.l.
Ec.
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Ec
6
C.l.
Ec.
Vp.l.
Fe.m.
Col.
Ec.
A.c.
7

Amblystoma upon the outer surface of the membrane at a corresponding stage of development (fig. 7). Continuous growth of the isthmus and the resulting bars produce the entire definitive plate. Frequently isolated centers of chondrification appear which fuse with other centers and all finally with the plate proper to produce the definitive structure. With regard to the growth of the fenestral plate into the membrane, one observation is important: the advancing edge is thin and the newly formed cartilage is always within the membrane and usually at its very middle (fig. 6).

The original invading bars of cartilage and isolated centers gradually extend in all directions and eventually meet and fuse. In this way there is produced, in larvae of 43 mm., the continuous plate shown in figure 26 where the chief difference in extent from that in the adult is its failure as yet to fill the whole fenestra. The stylus columellae has the appearance and relations of a rod which is secondarily applied to the surface of the fenestral plate rather than primarily a part of the latter's own substance. Even in the older stages where both elements are ossified, the distinction is evident at certain levels through the loose relations between the two elements (fig. 1).

The perilymphatic prominence is formed by the outpocketing of the fenestral membrane long before it becomes chondrified, but the M. opercularis is not in evidence until the transformation period arrives.

Fig. 5 Transection through the fenestra vestibuli of a larval Spelerpes bislineatus 31 mm. long. This section is caudad of that in figure 4. *A.c.*, arteria carotis interna; *C.l.*, canalis lateralis; *Ec.*, ear capsule; *Fe.m.*, fenestral membrane; *Op.*, fenestral plate (operculum) forming within the fenestral membrane; *V.p.l.*, vena petrosolateralis.

Fig. 6 Transection through the caudal portion of the fenestra vestibuli of a larval Spelerpes bislineatus 31 mm. long. *A.c.*, arteria carotis interna; *Cl.*, canalis lateralis; *Ec.*, ear capsule; *Fe.m.*, fenestral membrane; *Op.*, fenestral plate (operculum); *V.p.l.*, vena petrosolateralis.

Fig. 7 Transection through the fenestra vestibuli of a larval Amblystoma punctatum 35 mm. long. *A.c.*, arteria carotis interna; *C.l.*, canalis lateralis; *Col.*, columella, plate-like and spreading over the outer surface of the fenestral membrane; *Ec.*, ear capsule; *Fe.m.*, fenestral membrane; *V.p.l.*, vena petrosolateralis.

The further development of the fenestral plate is concerned with its ossification and has little or nothing to do with its morphology. As mentioned above, the stylus first chondrifies at those points where it comes into contact with the fenestral plate, and from here cartilage gradually extends cephalad until the long dense cord of the columellar proton is transformed into a cartilaginous rod, except for a short ligament which effects the attachment to the squamosum. Ossification in the stylus begins at its fenestral end and proceeds toward the suspensorial attachment, as did the chondrification, forming, at first, a shell of bone about a cartilaginous core which gradually diminishes as the bone increases in thickness. In the oldest adults studied cartilage still persists at the center.

Ossification of the fenestral plate begins on the ectal surface at the base of the stylus. From this as a center bony tissue spreads toward the periphery and finally the cartilage is entirely replaced by bone, the formation of which gradually extends from the ectal, through the plate, to the ental surface. In Amblystoma the ectal and ental plates of bone are formed independently.

From the foregoing paragraphs the morphology of the fenestral elements in Spelerpes bislineatus is evident. A brief summary of the morphology and development of these structures in Amblystoma will serve as a basis of comparison in the two forms. In Amblystoma the columella arises as a dense strand of tissue, extending between the ventral edge of the squamosum and the fenestral opening, opposite which the end of the cord of cells expands as the first step in the formation of a plate-like columella which is later completely to fill the fenestral opening. Throughout the developmental period and late into larval life its position outside the fenestral membrane is evident, as shown in figure 7. In late larval life the cephalic and dorsal margins of the plate become included within the membrane, preparatory to fusion with the ear capsule. Whether this fusion is effected by the active growth of the columella or of the lips of the fenestra, has nothing to do with the morphology of the element. Chondrification begins early, and gradually the plate of

newly formed cartilage is pressed against the fenestral membrane and, through growth at its periphery, extends over the ectal surface of the membrane and fills the fenestra. The primary fenestra being thus occupied at transformation, the second element or operculum is cut out from the walls of the ear capsule, which process forms and fills at one and the same time the secondary fenestra. The stylus in Amblystoma begins as a conical projection from the plate proper and extends toward the squamosum, following the original cord of cells, the unchondrified portion of which becomes the ligamentum squamoso-columellare. A comparison of the fenestral structures in Amblystoma and Spelerpes is facilitated if two points are borne in mind: 1) that the columella of the former is entirely extraotic in origin and, though plate-like, the whole structure is formed through its own growth without the addition of tissue from other sources; 2) the fenestra is small and almost completely filled in the mature larva by the columella. This influences, without doubt, the method of development of the operculum which is a part of the tissue of the ear capsule cut out into a distinct element.

In the method of the formation of the plate itself in Spelerpes lies the explanation of the homology of this fenestral element in the Plethodontidae and others to be mentioned later. The primitive cord of cells extending from the ventral edge of the squamosum to the fenestra possesses the same relations to facial nerve and to the arteria carotis and the vena petrosolateralis as in Amblystoma. This structure represents the columella of Amblystoma, but does not spread out into a plate when it comes in contact with the fenestral membrane. It remains a cylindrical rod taking no part in the formation of other structures in the sound-transmitting apparatus. It alone is extraotic in origin, and therefore represents the columella of Amblystoma. During development it becomes attached to the fenestral plate secondarily. The plate itself results from the growth of the isthmus fenestralis into the membrane and is, therefore, composed of cells derived from the ear capsule, and hence represents the operculum of the Amblystomidae.

While the development of the sound-transmitting apparatus in Spelerpes bislineatus shows many differences of detail from that of Amblystoma, it conforms, nevertheless, to the statement made by Kingsbury and Reed regarding the nature of this apparatus in urodeles generally. That is, there are two distinct elements present. The growth of the fenestral plate into the membrane from the lips, or its independent formation there, does not argue against its interpretation as operculum. In this connection one observes that in Triton and Diemictylus the operculum is formed partly by the cutting-out process and partly by growth of the cartilage into the membrane. The caudal part of this plate in Spelerpes possesses the same relations to parts of the internal ear as the operculum of Amblystoma. Furthermore, some time before the formation of cartilage in the caudal portion, the membrane bulges out in a fashion characteristic of the perilymphatic prominence of the operculum. It should be noted, too, that the extreme cephalic portion of the fenestra is not filled by the plate. This is evident in the drawing of the model (fig. 26). Perhaps a distinction should be made between the cephalic and caudal halves of the plate, but since it is composed entirely of tissue that belongs to the ear capsule itself, the term 'operculum' will be employed for the entire structure less the stylus which is morphologically 'columella,' as stated above. To the extent that the stylus at its caudal end becomes joined to the fenestral plate, this single element in Spelerpes represents a fusion of columella and operculum. A fusion of these elements in Amblystoma would result in an equal contribution to the plate on the part of both columella and operculum, while in Spelerpes the columella takes no part in the formation of the plate whatever.

There is a peculiar relation between the sound-transmitting apparatus and the hyomandibular cleft in Spelerpes bislineatus which should be mentioned. This relation exists from the very beginning of the columella to the assumption of terrestrial life. Mention has already been made of the dorsal extension of the hyomandibular cleft throughout embryonic life and its relation to the columellar proton. With the beginning of larval life the

dorsal extension of the cleft is reduced to an open and slight evagination of the oral epithelium toward the margin of the fenestra, where it takes up a position between the artery and vein and comes into actual contact with the perichondrium of the developing isthmus fenestralis and the fenestral membrane (figs. 8 and 9). At the same time it is connected by a thin sheet of fascia with the columellar proton. In older stages it loses a close connection with the fenestral plate, but retains its relations with the stylus columellae which has become chondrified (fig. 4).

A comparison of Amblystoma and Spelerpes larvae with regard to the relations of the fenestral elements and the hyomandibular cleft appears as a favorable argument for the belief that in Spelerpes the persistence and relations of the cleft are closely associated with a function, aside from any morphological significance which they may have. Without close observation and experimentation it is difficult to form a definite opinion of what that function may be. Judging from the nature of the relations, it might be of use, either as an aid in the apprehension and deglutition of food, or in the detection of disturbances in the water. Comparative evidence favors the latter. In Amblystoma larvae no such relations between oral epithelium and fenestral structures exist. Correlated with that condition it is noteworthy that the columella in amblystomid forms not only arises, but chondrifies, early in development, so that it is able to function as soon as an active free life begins. On the other hand, in Spelerpes the fenestral structures, especially the stylus, are tardy in their development. The larvae are active free-swimming organisms long before a well-defined and functional columella places the inner ear in communication with the suspensorium. This, together with the early connection of the cleft-vestige with the end of the growing isthmus and its later relation to the stylus, are significant. Of further interest in this connection are the observations of Bruner ('14) which would indicate that the water in the mouth forms a means for the transmission of disturbances between the environment and the inner ear. The larval period of this species is, at least, two

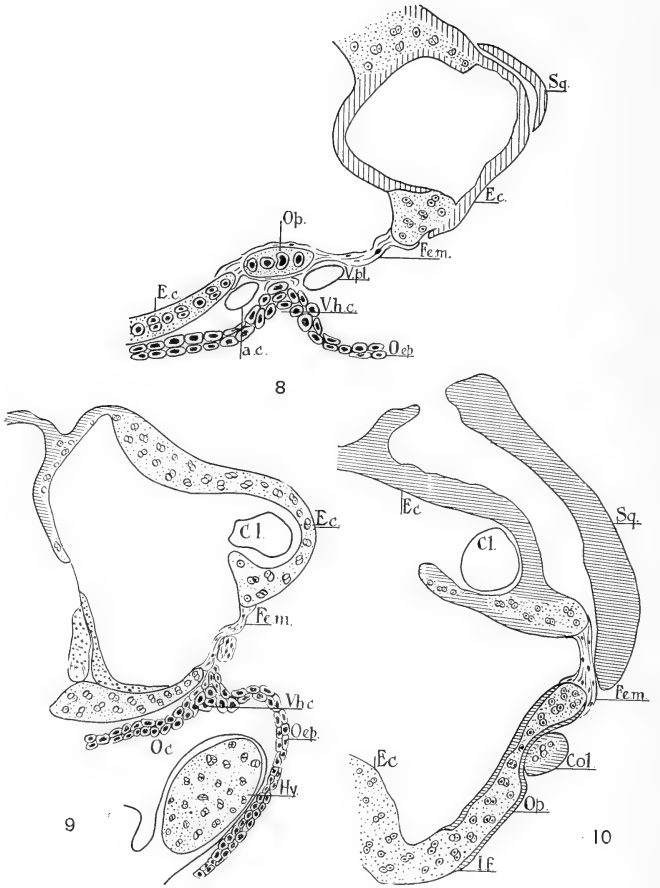


Fig. 8 Transection through the fenestra vestibuli of a larval Spelerpes bislineatus 23 mm. long. *A.c.*, arteria carotis interna; *E.c.*, ear capsule; *Fe.m.*, fenestral membrane; *O.ep.*, oral epithelium; *Op.*, fenestral plate (operculum); *Sq.*, squamosum; *V.h.c.*, vestige of the hyomandibular cleft; *V.p.l.*, vena petrosolateralis.

Fig. 9 Transection through the cephalic half of the fenestra vestibuli of a larval Spelerpes bislineatus 19 mm. long. *Cl.*, canalis lateralis; *Ec.*, ear capsule; *Fe.m.*, fenestral membrane; *Hy.*, hyoid; *O.c.*, oral cavity; *O.ep.*, oral epithelium; *V.h.c.*, vestige of hyomandibular cleft.

Fig. 10 Transection through the cephalic half of the fenestra vestibuli of an adult Gyrinophilus porphyriticus. *Cl.*, canalis lateralis; *Col.*, stylus columellae (columella); *Ec.*, ear capsule; *Fe.m.*, fenestral membrane; *I.f.*, isthmus fenestralis; *Op.*, fenestral plate (operculum); *Sq.*, squamosum.

years as compared with a few weeks in Amblystoma. During this period they inhabit cool streams, with more or less current, rather than stagnant pools, and are more buoyed up by the water. During late larval life the relation of stylus and cleft is gradually lost, so that at transformation all traces of former connections have disappeared.

Spelerpes ruber. This species has not been studied with the same detail as S. bislineatus. Larvae from 41 mm. to adults indicate that the relations and morphology of the fenestral elements are identical, except for certain specific variations. In this species the stylus is placed farther dorsad and not quite so firmly joined to the plate, a condition which foreshadows the relation of parts in Gyrinophilus to be discussed next.

Gyrinophilus porphyriticus. Both the fenestral plate and the fenestra itself in Gyrinophilus are nearly circular, in decided contrast to the elongate structures of Spelerpes. The plethodontid mode of ossification is followed. Although larval stages were considered, an examination of older larvae and adults alone is conclusive in the light of the development of these parts in S. bislineatus. There is no pronounced perilymphatic prominence as such. The whole fenestral plate represents an outpocketing away from the fenestral lips which, on the dorsal side, form a conspicuous ledge. The M. opercularis is attached at the middle of the plate, from which there is a conical projection into the muscle (fig. 32). The cephalic portion of the fenestra is not completely filled, a similarity of detail which this genus bears to Spelerpes. The space between the dorsal edge of the plate and the lips of the fenestra is filled to a certain extent by the dorsal growth of the plate itself, but to a greater extent by the development of the above-mentioned ledge. The downward and backward growth of the squamosal conceals some of the space which persists in this region.

The relation of the stylus to the plate is a much less close one than in any of the Spelerpes group. In the adult it is a rod lying against the plate, and sections show that, although it touches the plate, there is no fusion until the very tip end of the stylus is reached. Figures 32 and 10 illustrate this point.

Although the general relations of the stylus are the usual ones among urodeles, the course of the facial nerve deserves notice, since it varies in certain details from those in other species, and this variation is significant when compared with forms to be discussed later. This nerve issues below the stylus, which is normal. Figure 19 is a diagrammatic representation of the course of the ramus jugularis VII in Amblystoma. It leaves the main trunk just underneath the stylus and maintains a horizontal course across the lower third of the columella and operculum. In general, in its relation to fenestral structures, it may be said to occupy a ventral position. The course of the jugular branch in Gyrinophilus is illustrated in figure 22. Here the R. jugularis VII leaves the main trunk in a dorsal direction and keeps to a course along the dorsal edge of the fenestral elements.

A complete description of the development of the sound-transmitting apparatus in Gyrinophilus would be a needless repetition after what has already been written of Spelerpes. A larva 82 mm. long exhibits all of the essential features for an understanding of the morphology of the parts under consideration. There are shown by a comparison of figure 25 of Spelerpes and figure 31 of Gyrinophilus. The latter shows the isthmus fenestralis as present and in its normal position. The estate of the plate as a whole at this stage leaves no doubt as to the method of cartilaginous invasion of the fenestral membrane from the isthmus. A dorsal arm (*D.a.*) extends toward the stylus and a ventral arm (*V.a.*) extends caudodorsad. The indented margins and fenestrae within the plate point to its formation by growth and extension of the original invading arms and their fusion with isolated areas of independently formed cartilage within the fenestral membrane itself. The loose relation of the stylus and plate in the definitive state is here accounted for; only the extreme caudal end of the stylus comes into contact with the plate early enough to admit of fusion, both because of its morphological distinctness and an unchondrified area in the fenestral membrane underneath it which persists into late larval life. The long larval period here, as in Spelerpes, is associated with tardiness in the development of these parts.

The morphology of the sound-transmitting apparatus in Gyrinophilus is obviously the same as in Spelerpes.

Manculus quadridigitatus. In this species, so far as one can judge from a study of the adult only, the plethodontid type of sound-transmitting apparatus prevails. It appears that the dorsal arm from the isthmus extends scarcely dorsad of the stylus and that independent islands of cartilage do not form during the developmental period, so that the membrane is somewhat free in its dorsal extent.

Hemidactylium is similar in all respects to Manculus. The stylus, although firmly coossified with the fenestral plate, exhibits a loose morphologic relation, recalling those in Gyrinophilus. The two elements touch each other for a short distance and bony tissue forms between them, but the identity of each is clear at all levels.

Batrachoseps. Among all the Plethodontidae examined Batrachoseps is unique in its sound-transmitting apparatus. Of the three characteristic features of this apparatus in the Plethodontidae only one is evident in the adult. There is not the slightest suggestion of a stylus or of a suspensorial connection through a special cord of fascia. The isthmus fenestralis is absent, leaving the plate freely suspended in the membrane. There is, however, a well-developed M. opercularis. Furthermore, the plate is relatively short in its horizontal diameter. Except for features of ossification in the capsule and plate itself, the whole region might easily be identified as belonging to an amblystomid form in which only the operculum is present. Judging the structure and relations of this element from the adult only, it appears to represent the definitive operculum of Amblystoma. If this be true, its whole estate should be looked upon as the parallel of what occurs in Triton and Diemictylus. The available evidence points to such a conclusion. The relation of this element to other cranial structures favors its designation as operculum, although these relations might vary with such changes as the elongation, flattening, or shortening of the head. One relation which is here to be more relied upon is that with parts of the internal ear. A survey of the position of the colum-

ella and operculum, or their corresponding parts, in the whole series of caudate forms reveals a fairly uniform relation of the sound-transmitting organs to parts of the membranous ear. The operculum occupies a position directly opposite the caudal elements of the inner ear. It never extends farther cephalad than the caudal half of the lagena cochleae, thus including the extreme caudal extent of the sacculus only. Cross-sections in some cases do not contain the sacculus at all. The whole plate in Batrachoseps attenuatus is entirely caudad of the sacculus. It appears evident that the columellar element is absent in this form, and that which is present represents the operculum of other forms. The uniform conditions and mode of development of the fenestral structures in the Plethodontidae would not suggest a mode of development and morphology different in fundamentals for a particular genus. Taking this view, it appears that the columella (stylus) has failed to develop or at least to reach the definitive state. The slight relations of the stylus and plate in all plethodontids are in line with such an explanation. Batrachoseps is a strictly terrestrial species. If, as is the case with others of its family living under similar conditions, the larval state is passed within the egg, there is no need of a columella, and quite naturally it should disappear. In the adult a strong cord of tissue extends from the ear capsule between the artery and vein of the region to the squamosum which may represent the vestigial columella. The absence of the isthmus points to cartilage formed within the fenestral membrane as the source of the plate which becomes functional in the adult.

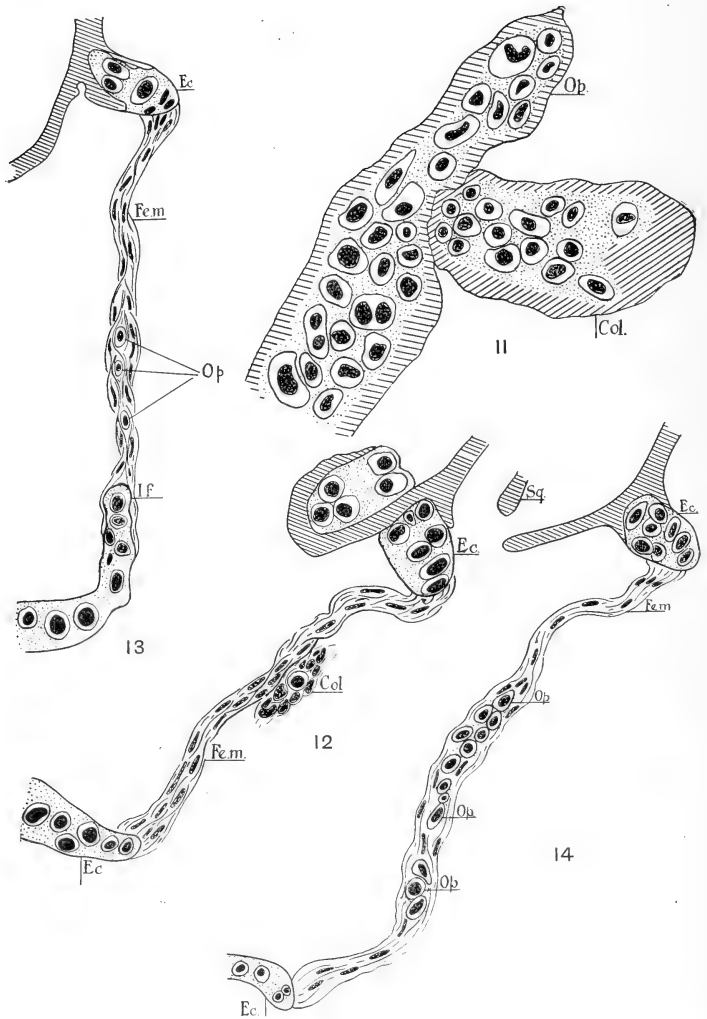
If these deductions prove true, then Batrachoseps represents the extreme in specialization of sound-transmitting apparatus among the Plethodontidae.

FAMILY DESMOGNATHIDAE

Desmognathus fusca, of all the plethodontid types studied, shows most clearly the independent origin and definitive state of the stylus and fenestral plate. In the morphology and relation of parts there is perfect agreement with Spelerpes, and the development is such that no doubt is left with regard to the

significance of structure. In certain minor details Desmognathus resembles Gyrinophilus more than other Plethodontidae, but in others it reflects conditions found in Plethodon. Concerning Desmognathus, Kingsbury and Reed wrote: "An examination of young larvae and embryos which would determine the origin or origins of the stylus and fenestral plate has not been undertaken. The mode of insertion of the stylus upon the fenestral plate, ... might suggest that the stylus alone developed outside the otic capsule as the description of Parker would indicate." A study of the development substantiates this statement and proves the correctness of Parker's views. As detailed a study as possible of Desmognathus was undertaken, because, at the time the work was begun, it was believed to represent a distinct family. A careful consideration of the group is justified, since it has strengthened conclusions regarding the relationships of this family, to be mentioned later, and aids in interpreting the general nature of these parts in Spelerpes, where they are found in a more generalized state.

An examination of the insertion of the stylus upon the plate gives the impression of a knob pressed into plastic material to which it adheres. In medium-sized adults the two structures are easily distinguished in sections, as shown in figure 11. The columella (stylus) arises in the typical way as a cord of cells independent of the ear capsule. It is not yet chondrified in larvae 26 mm. long. The cord of cells, however, is very distinct (fig. 12) and lies against the fenestral membrane for about 40 μ, when it ends abruptly. In this stage the isthmus fenestralis has extended some distance into the membrane, and isolated centers of chondrification, consisting of scattered cells, appear in the middle and caudal parts of the membrane. These cells are formed within the membrane, with no relations to extraotic elements. Figure 13, from a transection 40 μ caudad of the end of the columellar cord and through the apex of the isthmus, shows the position and relation of the cells just mentioned. Still further caudad chondrification has begun at several distinct places (fig. 14). The areas of cartilage are not yet associated with others at any level, but show growth in every direction and the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formation of a matrix. In larvae 27 mm. long the stylus is chondrified for nearly its whole length and is loosely joined to the cartilage of the rapidly forming plate.

The origin of the elements in Desmognathus is identical with those of the Spelerpes group, but the rôle of the isthmus is not so important. Figure 30 illustrates the mode of development. The isthmus, instead of extending directly upward and sending out both dorsal and caudal arms, thus effecting a union between it and the stylus, sends out only the caudal arm which grows diagonally upward and backward, never coming in contact with the stylus at all. Sooner or later it meets isolated centers of cartilage with which it fuses, and these centers in turn fuse with each other, thus establishing a band of cartilage between the original isthmus and the independently formed stylus. This band, when finally completed by the fusion of isthmus and isolated centers, extends to the caudal margin of the fenestra, where it bends upon itself, sending a bar of cartilage cephalad to join the end of the stylus as mentioned above. The place of fusion is marked *F* in figure 30. The fenestral bar is well outlined in larvae 26 mm. long, but an unchondrified space exists between it and the columellar cord of cells. Gradually these spaces are filled by growth, but certain areas remain open until filled by bony tissue.

By way of summary, it may be stated that in Desmognathus the plan of development and morphology of parts conform strictly

Fig. 11 Transection through the fenestral plate and stylus of an adult Desmognathus fusca. *Col.*, stylus columellae (columella); *Op.*, operculum (fenestral plate).

Fig. 12 Transection through the fenestra vestibuli of a larval Desmognathus fusca 26 mm. long. *Col.*, columellar proton; *Ec.*, ear capsule; *Fe.m.*, fenestral membrane; *Sq.*, squamosum.

Fig. 13 Transection through the middle of the fenestra of a larval Desmognathus fusca 26 mm. long. *Ec.*, ear capsule; *Fe.m.*, fenestral membrane; *I.f.*, isthmus fenestralis; *Op.*, isolated cartilage cells which through growth and fusion with others form the fenestral plate or operculum.

Fig. 14 Transection through the fenestra of a Desmognathus fusca larva at a level slightly caudad of that of figure 13. Length of specimen, 26 mm. *Ec.*, ear capsule; *Fe.m.*, fenestral membrane; *Op.*, fenestral plate (operculum) formed by the fusion of isolated areas of chondrification.

to those of Spelerpes, except that the fenestral plate is formed in a less degree from the invading isthmus and more from cartilage appearing independently within the fenestral membrane itself. Other genera of the family Plethodontidae have been studied and found to agree in all fundamentals with Spelerpes. As comparisons with Desmognathus and Spelerpes the observations of Peter ('98) are both interesting and significant. His studies were made upon Ichthyophis glutinosus, one of the Gymnophiona. He concluded that the sound-transmitting apparatus in this species represents two distinct components, one otic, the other extraotic in origin. The latter is first laid down as a continuous and dense cord of cells extending from the quadrate cartilage to the fenestral region. Chondrification begins at the fenestral end and proceeds toward the quadrate. The fenestral element in the stages figured by Peter is joined to the stylus, although in earlier stages the two are distinct according to descriptions. In the adult state, as shown by the descriptions and figures of the Sarasins ('87 to '93), the plate completely fills the fenestra. Peter concludes that the element in the fenestral membrane is to be likened to the operculum, and that the lateral projection or stylus represents the columella of urodele types. With regard to the presence of two elements in Ichthyophis and their independent origin, there is complete agreement with the Caudata.

FAMILY AMPHIUMIDAE

The fully formed fenestral element in Amphiuma is of the single-plate type with a large stylus. A typical isthmus fenestralis is present. Kingsbury and Reed described this structure in the adult, and according to material available at that time, were unable to make any statement regarding homology other than that the plate appeared to represent the columella. More recently additional material has been examined revealing new facts which should be presented. For the nature of the plate in the definitive state, see figure 29.

Embryos of a given stage in development of this species are relatively much longer than those of other urodeles, and the length of an embryo or larva does not serve as an index of development when compared with other urodeles.

The youngest stage studied was an embryo 30 mm. long, in which the ear capsule is completely chondrified. The sound-transmitting apparatus presents a condition not met with in other urodeles. The stylus is present and chondrified throughout its whole length while as yet no fenestral plate is formed (fig. 27). A section through this region (fig. 15) shows the chondri-

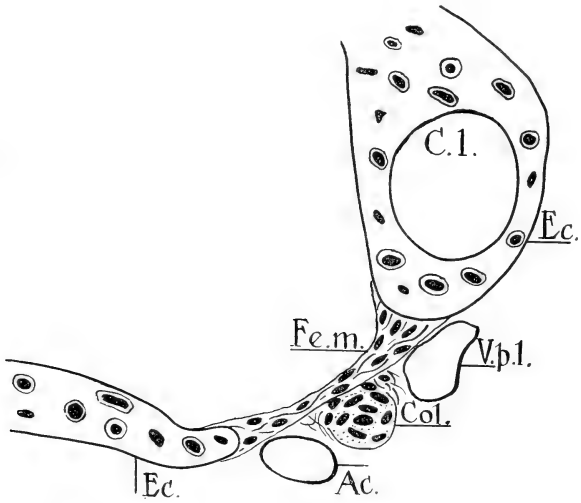

Fig. 15 Transection through the fenestra vestibuli of an embryo Amphiuma means 30 mm. long. *Ac.*, arteria carotis interna: *Cl.*, canalis lateralis; *Col.*, stylus columellae (columella); *Ec.*, ear capsule; *Fe.m.*, fenestral membrane; *Vp.l.*, vena petrosolateralis.

fied stylus lying along the fenestral membrane, which here, as at all levels, entirely lacks cartilage. The progress of chondrification, so far as direct observation is concerned, remains unknown, since stages of the proper age were not available. The stylus extends only a few microns along the fenestra. Two series of embryos 33 mm. long (which vary as regards advance in development) give a clue as to one mode of plate formation,

that of the growth of cartilage from the stylus out upon the membrane of the fenestra. Growth is confined entirely to the dorsal side of the stylus and its tip end. A comparison of figures 15 and 16 illustrate this point. In the former is the very beginning

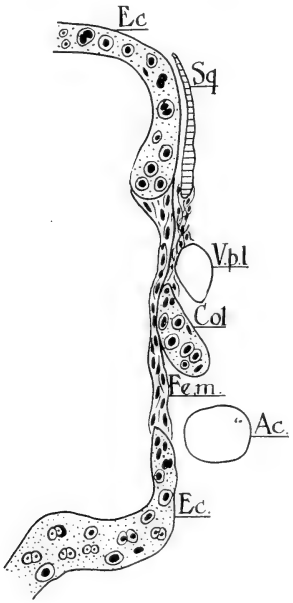

Fig. 16 Transection through the fenestra vestibuli of an embryo Amphiuma means 30 mm. long, but more advanced than that from which figure 15 was drawn. *Ac.*, arteria carotis interna; *Col.*, stylus columellae (columella) spreading slightly over the outer surface of the fenestral membrane; *Ec.*, ear capsule; *Fe.m.*, fenestral membrane; *Sq.*, squamosum; *V.p.l.*, vena petrosolateralis.

of this growth, where, in the section sketched, only one cell is shown pushing out toward the membrane. Figure 16, which is more advanced, shows that the diameter of the stylus is much increased, all of which is due to the extension of its own substance over the surface of the membrane. To what extent the fenestral

plate is formed by such growth cannot be definitely stated, but certain limits for its contribution may be judged by two observations upon a young adult 85 mm. long in which the plate is not yet completed. The first point to be noted is that the staining reaction of the stylar and fenestral cartilage is different and apparently consistent in each. From this it appears that only a small portion of the plate surrounding the end of the stylus is formed by the spreading of the stylar tissue over the fenestral membrane and this only about the very end of the stylus. The second observation of importance is that concerned with the connection of the fenestral plate and otic capsule. An isthmus fenestralis is present, typical in location and relations.

In urodeles there are only two different connections between the fenestral plate and the margin of the fenestra. One of these is typified by the Amblystomidae, the other by the Plethodontidae, both described above and determined by development. In the amblystomids the connection is produced by growth from the plate toward the ear capsule, in the latter by growth from the ear capsule into the fenestral membrane. The two are distinct as regards location and mode of development and are consistent within the groups in which they obtain. The evidence, so far as it may be considered as such, favors the conclusion that the isthmus in Amphiuma is of the plethodontid type. Further evidence for this view is found in the incomplete state of the plate in the young adult studied. The band of cartilage below the stylus (fig. 28, *V.a.*) represents the growing ventral arm from the isthmus, while that above is to be considered the dorsal arm as in Spelerpes. Except for the few developmental observations actually made and the conclusions deduced from comparisons with other urodeles, the columellar nature of the fenestral plate in Amphiuma would not be questioned. All that has been gained from the present study forces the conclusion that the sound-transmitting apparatus of this species resembles the plethodontid type more than the amblystomid type, and in this respect Amphiuma must be viewed as intermediate between Necturus and the Plethodontidae.

FAMILY NECTURIDAE

The sound-transmitting apparatus has been discussed at sufficient length in another communication (Reed, '15); therefore, only a summary is given as an aid to comparisons made later in this paper. The columella appears in the usual fashion outside the ear capsule and the proton comes to lie for a short distance along the fenestral membrane. As chondrification advances, the tip of the stlyus spreads over the fenestral membrane, but to a very limited extent as compared with Amblystoma. Approximately a third of the plate, located in the cephalic part of the fenestra, is formed in this manner. This area is triangular in outline, the base being applied to the cephalic lips of the fenestra while the apex is on a level with, and extends slightly beyond the stylus. Other parts of the plate are formed by cartilage, produced within the membrane itself, and during growth it becomes joined to that proliferating from the stylus (fig. 33). The freedom of the definitive plate from the lips of the fenestra and its formation partly from extraotic and partly from otic tissues stamp it very decidedly as an intermediate in comparison with the amblystomid and plethodontid types.

FAMILY TYPHLOMOLGIDAE

The general estate of the sound-transmitting apparatus in Typhlomolge has been described by Kingsbury and Reed. The serial sections of the specimen 95 mm. long have been carefully reexamined in the light of what has been gained through developmental studies of other species. The presence of an isthmus fenestralis indicates that the fenestral plate is of the plethodontid type. In connection with this, as the best available evidence, certain features are mentioned here for whatever value they may have in a significant way. These features could not be depended upon were it not for the consistent rôle of the isthmus in all cases where it has been possible to trace its development. The isthmus, projecting into the fenestral membrane, very soon extends underneath a bony plate to which it becomes attached through cartilage cells, as shown in figure 17. The cartilage of

the isthmus is within the membrane, while that of the small plate rests against it. These are the relations which should obtain, respectively, for elements of otic and extraotic origin. In following sections caudad, this relation is found to persist. The extension of the extraotic part of the plate in the dorsal direction is only slightly above the base of the stylus. Figure 18 shows this feature and also that the stylus appears to spread out

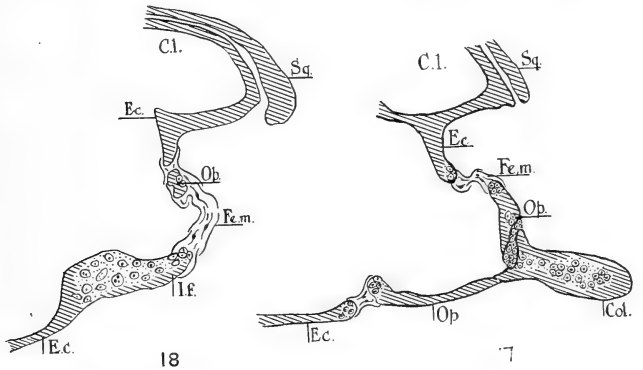

Fig. 17 Transection through the fenestra vestibuli of an adult Typhlomolge at the level of the stylus columellae. *C.l.*, canalis lateralis; *Col.*, stylus columellae (columella); *Ec.*, ear capsule; *Fe.m.*, fenestral membrane; *Op.*, fenestral plate (operculum); *Sq.*, squamosum.

Fig. 18 Transection of the ear region of Typhlomolge, passing through the fenestra vestibuli caudad of the level of the stylus columellae. *C.l.*, canalis lateralis; *Ec.*, ear capsule; *Fe.m.*, fenestral membrane; *I.f.*, isthmus fenestralis, continuous with the lips of the fenestra; *Op.*, fenestral plate (operculum), the free membrane between it and the dorsal margin of the isthmus representing the space into which the columella failed to spread; *Sq.*, squamosum.

slightly as a funnel-shaped element. Taking together what evidence there is, both direct and deduced, it appears that the fenestral plate is double; the stylus and a small portion of the plate being extraotic, and, therefore, columella, while the isthmus and bulk of the plate represents tissue formed within the membrane and, therefore, otic in nature. In principle it agrees with the plethodontid type.

FAMILY SIRENIDAE

The possible conclusions regarding the fenestral elements in Siren are based to a minor degree upon circumstantial evidence, which, however, carries weight with one who has reviewed these structures in the entire series of caudate amphibia. The known facts may be briefly summarized. There is only a single element in the fenestra, and this is not connected with either quadrate or squamosal. In individuals up to 215 mm. in length the plate is connected with the ear capsule in the dorsal part of the fenestra (fig. 34). This connection is different from the cephalic connection of the columella in the Amblystomidae (fig. 20) and the ventrocephalic relations of the plate in the Plethodontidae (fig. 22). In a specimen 440 mm. long (undoubtedly a fully matured individual) the fenestral plate is free from any cartilaginous or bony connections with the ear capsule whatever (fig. 35). Cope ('88), writing of this structure, observes: "In Siren the stapes is osseus. Its columella is replaced by the stapedius muscle which extends posteriorly." In view of these facts, the origin and nature of the fenestral element in Siren becomes clear. Its freedom from the ear capsule in the definitive state, its lack of connections with the suspensorium, and the presence of the M. operculare (stapedius muscle of Cope) identify it as the homologue of the operculum of the amblystomid forms. Not only does it possess the relations of the operculum, but it is formed from the walls of the ear capsule by the 'cutting out' process as described by Kingsbury and Reed for this element in Amblystoma. In a specimen 133 mm. long (fig. 34) the 'uncut' portion of the opercular margin is coextensive with its width, and there are only slight relations with the M. operculare. Serial sections of the head of a specimen 215 mm. long show the uncut portion very much reduced, with the M. operculare firmly attached to the side of the plate along with the strong ligamentum hyocolumellare.

There is no evidence as to the fate of the columella in this species. In others, where this element has become functionless in adult life, it has fused with the cephalic lips of the fenestra,

though retaining its stylus. In those where total disappearance obtains it has come about through loss of stylus and complete incorporation with the ear capsule. Inferentially, this accounts for the complete effacement of the columella in Siren. Leaving inference, however, out of the question, and drawing upon the known facts only, it becomes obvious that the fenestral plate in Siren is identical with the functional operculum of the adult Amblystoma and, being such in its morphology and the apparatus as a whole lacking the columellar portion, it argues for the similarity of the sound-transmitting apparatus of Siren to that of Triton and Diemictylus.

SUMMARY

A general survey of the sound-transmitting apparatus in the tailed Amphibia reveals the existence of two morphological types. One has been mentioned as the amblystomid type since it is found in its most generalized state in the Amblystomidae. The other is typified by the Plethodontidae and may, therefore, be termed the plethodontid type. In the perfect amblystomid type two distinct elements are present in the completed apparatus. One of these is columella which is extraotic in origin. It is composed of a stylus which joins the suspensorium at its cephalic end and at its caudal end spreads out over the fenestral membrane, forming a plate. This structure serves during larval life. At transformation the columella fuses with the ear capsule, and a new element, the operculum, is cut from the walls of the ear capsule to function during adult life. It occupies a position caudad of the columella and comes into connection with the cephalic end of the M. opercularis. The variation from the perfect or generalized type results from a loss of function of the columella when the terrestrial existence is assumed. The Amblystomidae and Salamandra possess this type in its most perfect state. The identification of the type resides in the operculum, it being the constant and consistent one of the two elements. In the direction of Triton and Diemictylus the columella develops, but soon completely disappears through fusion with the ear capsule, leaving only the operculum as a distinct element. So far as there

is any evidence, the only element in Siren is the operculum, the general state of affairs resembling those of Triton. The single element in Cryptobranchus is the columella, formed by the growth of the stylar portion over the fenestral membrane in true amblystomid fashion. The failure of the operculum to appear in this form is due probably to its aquatic life.

That which has been taken as the plethodontid type of sound-transmitting apparatus is exemplified by Spelerpes. The plate filling the fenestra is single and possesses three extrinsic connections: *a*) one with the suspensorium through the stylus; *b*) one with the shoulder-girdle through the M. opercularis, and, *c*) one with the ear capsule through the isthmus fenestralis. The plate itself is formed by both the growth of the isthmus into the fenestral membrane and the independent formation of cartilage within the substance of the membrane which meets and fuses with that of the invading isthmus. Figures 23, 24, 25, and 26 illustrate the mode of formation. The stylus is the only representative of what in the amblystomid type is columella. Variations from this type in its perfect state, as just described, are found in Necturus, Typhlomolge, and Amphiuma, where the stylus expands, forming a varying portion of the plate itself. In Batrachoseps, where the stylus (columella) has disappeared entirely, the isthmus is likewise absent, leaving a plate which is free from the ear capsule and which represents, apparently, one derived from independent cartilage formed in the membrane of the fenestra.

The characteristic features are the distinctness of the two elements, columella and operculum, in the amblystomid type and the fusion of the two in the plethodontid type as well as a difference in the exact mode of origin.

THE PHYLOGENETIC SIGNIFICANCE OF THE SOUND-TRANSMITTING APPARATUS

The following discussion is presented with a twofold object: first, to outline the history of the tailed amphibia as one reads it in the morphology of the sound-transmitting apparatus, and, second, to emphasize the zoological bearing of the subject as a

means of interpreting the morphology of the various systems of organs in these animals. The present discussion is not an attempt, primarily, to meddle with the classification.

It has been the aim in most instances to employ the same technical designation for the various groups and species of tailed amphibians as were used in previous publications upon this subject. An attempt on the part of systematists, however, to establish a stable nomenclature through the application of the rule of priority, together with views gained from more recent studies regarding the affinities of groups, have wrought many changes in names. In order to avoid the possibility of confusion, a comparative table of former names and those now considered correct is here introduced.

FORMER NAME	NOW EMPLOYED
Proteidae	Necturidae
Pleurodelidae	Included with the Salamandridae
Diemictylus	Notophthalmus
Amblystomidae	Amblystomidae
Amblystoma	Amblystoma
Amblystoma punctatum Cope	Amblystoma maculatum (Shaw)
Chondrotus tenebrosus Cope	Amblystoma tenebrosum Baird and Girard
Spelerpes bilineatus Cope	Eurycea bislineata (Green)
Autodax lugubris Cope	Aneides lugubris (Hallowell)
Desmognathidae	Included with the Plethodontidae

The studies embodied in the present paper, as well as its forerunners, have emphasized, in the mind of the writer, three points: *a*) that much of the confusion which has existed respecting the sound-transmitting apparatus of the Urodela has resulted from fragmentary studies, and that a true morphological interpretation of the structures in question is to be arrived at only through a complete developmental study of each distinct type

of auditory element found to exist in each taxonomic group; *b*) that the possibility of neoteny, as the explanation of certain features in such forms as Necturus, Cryptobranchus and others, cannot be overlooked, and, *c*) that the morphology of these organs has a significant bearing upon the relationships and descent of the principal groups of caudates.

It is contended by some that structures so intimately associated in function with the habits of the animal are likely to be affected by the environment and, therefore, unreliable in showing affinities or tracing descent. It is true that these structures are not only affected by the environment and needs of the animal, but show a decided adaptation in this respect. There are probably few internal structures in animals which are not ultimately affected by environment, and the differences of form and relations of internal parts are, to a considerable extent, a reflection of the life and habits of the animal itself. Form and relation of parts alone, therefore, do not always constitute reliable criteria for passing judgment, either with regard to morphology or lineage. It is exactly to this error that some of the confusion mentioned above can be traced. It has been apparent from the beginning that, so far as this system of organs is concerned, the only safe basis for homology resides in the principle that two structures, however much they may resemble each other in form and function, are different, unless comprising the same combination of elements. For example, the fenestral plate and stylus of Necturus and Cryptobranchus, in form and skeletal relations, appear identical. Development shows them to be entirely different in the elements which combine to form them, and they are, therefore, not homologous structures and not indicative of close affinity of the animals possessing them.

It is mentioned in the summary of the first part of this paper that two morphologic types of sound-transmitting apparatus occur in the tailed amphibia. When these types in the various groups are reviewed with the above-mentioned principle in mind, it will be found that the different families of Caudata become grouped into two legions, each of which represents a line of descent, judged by the nature of the variations of the combining

elements to form the definitive sound-transmitting apparatus. The forms included within a given legion possessing a sound-transmitting apparatus involving the same architectural principles are considered as more closely related to each other than to those of another legion.

Since the M. opercularis occurs in more than half of the urodele families, regardless of legion, and bears a direct relation to the mode of life of the animal, it is to be considered as of physiological rather than morphological import. This being its status, it is obviously of no significance in a strictly phyletic consideration. The whole question of descent and relationships of the urodeles, so far as concerns the sound-transmitting apparatus, hinges upon the elements composing the fenestral plate or plates and their mode of origin. Thus, in the Amblystomidae, Salamandra, Triton, and Diemictylus one finds the constant and independent operculum of the amblystomid type. They all agree in this particular among themselves and differ from all other groups. They may, therefore, be looked upon as of kin or having descended from a common ancestral stem in which this morphological feature was well established and survived the vicissitudes of change in such a way as to preserve its caste. Similarly constant in this legion is the tendency of the columella to spread over the outer surface of the fenestral membrane and form a plate which is continuous with the stylus columellae. The variations in the state of the columella in this legion appear to be correlated with the extent to which the various forms have become terrestrial. The tendency of the columella to fuse with the ear capsule expresses the trend of modifications with regard to this element in this legion. It is very apparent that the more terrestrial a species becomes, or the longer the period in its descent during which it has occupied the terrestrial zone, the more completely is the columella fused with the ear capsule. The extreme in this direction is found in Triton and Diemictylus, where complete effacement of the columella has taken place. The tritons, it will be noted, are terrestrial except at the breeding season. The aquatic abode of Diemictylus viridescens in the mature state has been secondarily acquired in more recent times, following a ter-

restrial period of existence, as pointed out by Gage ('91). The other extreme (the less modified) is found in the Amblystomidae and Salamandra, the latter in many respects exhibiting features which foreshadow the Triton state. The columella in this legion, although fusing with the ear capsule in varied degrees, shows, nevertheless, its characteristic spreading over the fenestral membrane during development. The plate thus formed does not fuse with the operculum or contribute to it in any way whatsoever. In no other caudates does this state obtain.

Although the columellar element in Siren is unknown, the morphology of the operculum is sufficient for the inclusion of this family among those of legion I. There are, however, certain features of habit and morphology which seem to be at variance. Thus it appears that the type of sound-transmitting apparatus found only in terrestrial urodeles is here present and well defined in an aquatic species. It seems desirable, therefore, to introduce whatever there may be of evidence upon this point gained from other studies upon this species. Cope ('85) was the first to point out that the present aquatic abode of Siren is secondary, following a period of terrestrial existence. His conclusion was based upon a tendency of the gills to disappear and become functionless in specimens of a certain age, reaching full development again only in large adult specimens. He writes: "The only explanation appears to me to be that the present sirens are the descendants of a terrestrial type of Batrachia, which passed through a metamorphosis like other members of their class, but that more recently they have adopted a permanently aquatic life, and have resumed their branchiae by reversion." In a later publication, Cope ('88) expressed the belief that he had found confirmatory evidence in the support of this view in the structure of the ossicula auditus. The morphology of the sound-transmitting apparatus as deciphered in the present study supports Cope's belief for an operculum, of whatever mode of formation, in communication with the shoulder-girdle, is associated only with a terrestrial existence, and, if its function has been correctly interpreted, is useful in such an environment only. The period of terrestrial existence of the antecedents of the present Siren must

have been extended and pronounced, for the columellar element, which is useless on land, has disappeared without leaving a trace in the adult. On the other hand, there are structural features which argue that this secondary aquatic period has been long enough to admit of certain readjustments of the sound-transmitting apparatus to this type of abode. Extending between the operculum and the hyoid is the extremely dense and large ligamentum hyo-operculare which places the inner ear in communication with the exterior as perfectly as could the columella itself. This ligament is by far the largest and most pronounced of any ligament in this region in any urodele. Although it is attached to the operculum, and is given a name which indicates its relations, it is quite likely that it represents the ligamentum hyocolumellare of other urodeles. The extent and relations of the general sheet of fascia in which it is formed admits of no other conclusion. Siren is the only urodele in which the operculum has such relations with the hyoid. The only interpretation of its relations here seems to be that of compensating the loss of the columella in transmitting disturbances from the surrounding water to the inner ear.

It seems probable that the morphology of the sound-transmitting apparatus in the Tritons and Sirens and their past history are identical; but this does not necessarily argue for a close relationship of these forms within the legion itself. Norris ('13), in his work upon the cranial nerves of Siren, refers to strong similarities between it and other urodeles, which is significant in the present consideration in two connections: one bears evidence in support of the view that Siren is not a primitive form, while the other points to a close relationship with those urodeles which comprise legion I as here constituted. Bearing upon this point in particular, in his summary Norris writes: "The contribution of maxillaris and buccalis fibers to the profundus palatine anastomosis has such a closely corresponding arrangement in Triton (Coghill) and also in Salamandra, if von Plessen and Rabinovicz's figures be correct, that it can hardly be explained as incidental."

It has been stated above that the complete absence of the columella in Siren points to an extended period of terrestrial existence further back in its phylogeny. A study of the general anatomy of this form by H. H. Wilder ('91) led him to the same conclusion. In summarizing his work he observes concerning the phylogenetic relations of Siren: "I am fully convinced that it has once possessed a terrestrial existence and been driven back to an aquatic life during the struggle for existence, similarly to the case of the Axolotl. . . . But unlike the Axolotl, which has simply repressed the later stages and represents still a fairly typical larva, the Siren-form has been modified by the influence of external conditions during a much longer period of time. . . ." While the position assigned Siren in plate 6 is tentative, although indicated by all of the available structural evidence, its position within the legion seems unquestionable.

The case of the Cryptobranchidae, so far as the sound-transmitting apparatus is concerned, must be adjusted by the evidence offered by the columella alone, since the operculum is wanting. The fenestral plate is single, possesses only suspensorial connections, and is formed by the spreading of extraotic material over the outer surface of the fenestral membrane, in which respect it agrees with the columella of the amblystomids, tritons, and Salamandra, and is at variance with every other known group of urodeles. This feature, being in such sharp contrast between the two great groups of urodeles, it very clearly allies the Cryptobranchidae with the amblystomid division or legion I. The position of this family within the legion is interpreted as a direct offshoot of the amblystomid stem.

The fenestral plate of the Cryptobranchidae with its suspensorial connections only, is of the type found in aquatic species or during the larval period of terrestrial forms and does not of itself indicate whether these animals are primitively or secondarily simple. During the transitional period from the fish-like to the terrestrial amphibians, the skeletal remains, and especially the restorations, denote a lumbering mode of locomotion in which the body was scarcely elevated above the substratum upon which it probably rested during inactive intervals. What

these movements must have been may be appreciated by recalling the waddling gait of a walking fish and the crawling gait of a modern salamander. The transitional amphibian must have possessed a mode of locomotion which would fall between these two extremes, in which case the jaws and branchial apparatus rested upon the substratum for a large portion of the time. The lateral line sense becoming functionless as the animal left the water, and hearing, if it existed in a refined state, certainly becoming much impaired, left these transitional amphibians with no special means of communication between the inner organism and disturbances which might occur in the surroundings. The relation of the jaws to the substratum, through contact, and to the auditory capsule, through the hyomandibular and its ligaments, formed a natural pathway for the transmission of vibrations. Thus, for physical reasons alone, it can be understood how the columella represents a refined hyomandibular and how the fenestra vestibuli may have come about. The structural supports for this view and the importance of such hyomandibular relations as are found in the notidanid sharks were discussed by Kingsbury and Reed ('09).

Accepting the general view that the larval period represents a more recent interpolation in the life-cycle of amphibians, one observes that the gait and relations of the body to the substratum of a modern larva is undoubtedly like that of the earliest amphibian forms, and that the demands upon the columella of the recent larvae are unquestionably no different from those made upon this structure in the primitive estate when amphibians were slowly evolving from fish-like forms. Since the columella was the first of the sound-transmitting elements to appear in phylogeny, it is quite natural that it should persist and function during the larval period of recent salamanders, although this period is a later addition to the life-cycle. It follows, then, that, in aquatic species such as the Cryptobranchidae the columella alone does not indicate the phyletic relations of its possessor, and one must look further into the morphology of these animals in order to determine their true rank.

Various studies of the Cryptobranchidae contribute information which is suggestive in its bearing upon the rank and kinship of this group. Versluys ('09), in summarizing the structure of the Cryptobranchidae, observes that they retain many features of a larva, and, at the same time, acquire many of adult salamanders. Thus the skull is that of the adult, while the circulatory organs approximate those of the larva. Versluys concludes with the statement that the Cryptobranchidae are to be looked upon as partly transformed larvae and an offshoot of the amblystomid stem. That they are arrested in transformation is the conclusion of Bruner ('14), based upon a study of the respiratory mechanism. Wiedersheim ('77) and Drüner ('01, '02) find that both the skull and branchial apparatus of the Cryptobranchidae bear striking resemblances to these structures in Raniceps and Hynobius, which argues for an amblystomid alliance of this family as shown by Wiedersheim in his phyletic arrangement of families. Herrick ('14), in a comparative study of the cerebellum of urodeles, discovered some suggestive points which I interpret as designating the Cryptobranchidae an offshoot of the amblystomid stem rather than the reverse of these conditions. In one instance Herrick writes: "The cerebellum of Cryptobranchus occupies an intermediate position between those of Amphiuma and Amblystoma." The position given the Cryptobranchidae in plate 6 is in accord with Herrick's observation.

A review of the important features of morphology, development, and distribution supports the view that the Cryptobranchidae are not primitive amphibians, but, as Smith ('12) has pointed out in his conclusions, are terrestrial forms which have secondarily become aquatic, in consequence of which certain larval features are retained, although these forms advance far enough toward their former state to actually begin transformation which is arrested. The conclusions arrived at from a consideration of the sound-transmitting apparatus are not in harmony with any view which allies the Cryptobranchidae with any other than the amblystomid group.

The sound-transmitting apparatus of the amblystomid legion seems to the mind of the present writer to echo the past history

of all its members. During the early transitional period, when the head must, for the greater part of the time, have rested upon the substratum, the columella became perfected and served as the communicating element between inner ear and jaws. As amphibians became more and more terrestrial the head and anterior end of the body was elevated above the substratum, in consequence of the perfection in the use of the arms in terrestrial locomotion. This elevation of the head rendered the columella functionless, and, a second time in their evolution, these animals were left without direct communication between the outer world and the inner ear. As a compensation for this loss a second auditory element, the operculum, was cut out from the ear capsule behind the columella; this appropriated a slip from the adjacent musculature, thus coming into communication with the shoulder-girdle and establishing a new coupling between the inner ear and the substratum along which disturbances might travel. The columella, being no longer of use, gradually fused with the ear capsule, leaving only the operculum freely suspended in the fenestra as the functional organ. The Amblystomidae either live in their original type of habitat or have departed only slightly from the terrestrial habitat of their ancestors, so that more of the primitive features of the sound-transmitting apparatus persist than in any other group. The Cryptobranchidae must have separated from the amblystomid stem fairly early, since sufficient time has elapsed for the complete suppression of the operculum. If this view be correct, the sound-transmitting apparatus of the Cryptobranchidae is secondarily simple and the Amblystomidae are, so far as the auditory structures are concerned, the most primitive of living Caudata. It might be argued that the Cryptobranchidae returned to an aquatic environment before the operculum had become a functional part of the sound-transmitting organs, in which event they would necessarily be looked upon as primitive urodeles. The nature of their partial transformation, however, and numerous features of structure do not encourage such a conclusion.

After the separation of the modern Cryptobranchidae and Amblystomidae from the main stem of this legion, all the other uro-

deles grouped here exhibit unmistakably the impression of terrestrial existence upon the sound-transmitting apparatus. The state of these organs in Salamandra is a parallel for that in the Amblystomidae, although a bit more inclined toward the terrestrial type. The Tritons, Diemictylus, and Siren, whatever their present-day habits, possess a type of sound-transmitting organ that could have become perfected only in the terrestrial zone. The columella has been a functionless structure for such a continued period that, in recent forms, it fails to produce a stylus or a permanent communication with the suspensorium, and at a very early period of development is effaced completely through fusion with the ear capsule, leaving the operculum only to communicate with the exterior through the M. opercularis and the arm. Diemictylus and Siren have returned to an aquatic abode so recently as still to retain the sound-transmitting apparatus in its former highly specialized state. They are, therefore, considered as having departed in this respect most widely from primitive conditions, and accordingly represent the culminating branches of the amblystomid stem or legion.

Legion II, the plethodontid group. The arrangement of this group is not fraught with as many difficulties as the former, since the morphological nature of the sound-transmitting organs is such that the generalized and specialized states are easily detected. This minimizes the difficulties of phyletic arrangement.

The distinctive peculiarities of the sound-transmitting apparatus of this legion are found in the presence of a single, but compound, fenestral element under all conditions of life and at all stages of development. The stylus is always present and is connected with the suspensorium. The variable element is the M. opercularis, which is present or absent, accordingly as the animal is terrestrial or aquatic in habit. The state of this muscle is precisely that which obtains among the forms of legion I. In the amblystomid legion the two elements, columella and operculum, are always distinct so far as fusions between them are concerned. In legion II, or that which is conveniently termed the plethodontid group, representatives of the columella and operculum fuse with each other in varying degrees, resulting in

a compound fenestral plate which is quite in contrast to the two distinct plates of species composing legion I, where the columella invariably fuses with the ear capsule when its function is reduced. This compound relation of elements represents one which is obviously a derivative from the more primitive estate of two distinct and simple elements and one which is reminiscent of a pronounced terrestrial life far back in phylogeny.

That the Necturidae are neotitic forms, and that they rank with others of the urodele order, has been pointed out by Kingsbury ('09), Norris ('11), Bruner ('14), and others. The morphology of the sound-transmitting apparatus harmonizes with such a view. This family is adjudged the most primitive of the legion since the fenestral plate is an exact intermediate between others of its group and those composing the amblystomid legion. The columella, during development, spreads out over the surface of the fenestral membrane so as to fill considerably less than half of the window, and fuses with opercular tissue formed within the fenestral membrane itself. This mode of formation reflects a former terrestrial existence for the Necturidae and may be explained by a brief reference to the mode of development of fenestral elements in Amblystoma and Triton. In the former the 'cutting out' process in the production of the operculum is a necessity resulting from the complete filling of the primary fenestra by the columella, which, in this form, comes into full function and proportions. In Triton the columella is so small because of its functionless state and it fuses so early with the lips of the fenestra that it forms merely a rim of cartilage, thus leaving a great portion of the primary fenestra free for the later invasion of cartilage, or for its formation within the free membrane. Consequently, in Triton, the operculum is produced, in part, by the cutting-out process, and, in part, by the growth of cartilage from the edges of the window into the fenestral membrane. The ultimate effect of this trend of modification, then, is the increase of free space in the primary fenestra, within which the opercular element may develop, with no necessity of 'cutting out' from the walls of the ear capsule. Thus one may account for the mode of development and morphology of the fenestral

plate in the Necturidae. As stated above, it points to a former terrestrial existence for these forms, in consequence of which the columella became reduced and fused with the rapidly forming operculum, rather than with the ear capsule. The loss of the M. opercularis is to be associated with the recently acquired aquatic habit. There results, in the Necturidae, a single fenestral plate which is perfectly free from the ear capsule and which bears the outward form of that structure in the Cryptobranchidae, but morphologically is quite different. The two families, judged by these organs, are not closely related. The Necturidae are considered as the most primitive of the plethodontid legion since the columella spreads out over the fenestral membrane to a greater extent than in any of the others of the group. This condition is interpreted as reminiscent of the earliest type of sound-transmitting apparatus. The family is, therefore, placed in plate 6 as an offshoot of the plethodontid stem, intermediate between others of that group and the amblystomid legion. Such a relationship is in harmony with the presence of lungs in the Necturidae which they should not possess were they closer of kin to the Plethodontidae. Especially significant in this connection are Norris' ('11) observations upon the cranial nerves, which are said, in their entirety, to reach a rather high degree of specialization, and, in their arrangement, to resemble both the plethodontid and amblystomid types and especially the former.

The other families of the plethodontid group represent branches which originate from a main stem leading back toward the place of departure of the Necturidae. In these families there is a gradual reduction of the columella from a state where it spreads slightly over the fenestral membrane, to one where, as in the Plethodontidae and Desmognathidae, it is represented by stylus only and takes no part whatever in the formation of the plate, to the surface of which it becomes joined. The extent to which the columella extends over the fenestral membrane is taken as the index of specialization. Applying this criterion, the Amphiumidae stand nearest the Necturidae, agreeing with them and differing from the others in the possession of lungs.

The Amphiumidae have been variously placed with regard to their kinship. Cope held tenaciously to the view that they represent a group intermediate between urodeles and the Apoda. The cousins Sarasin ('87 to '90) proceed a step further, and include the Amphiumidae among the Apoda. Kingsley ('02) made a critical study of the various supposed similarities of these groups and observes that it "would appear that some of them [resemblances] are of minor value, some are based upon imperfect knowledge or misconception, while some are false." Kingsley's study seems to have settled for all time the urodele affinities of the Amphiumidae. With that much granted, the sound-transmitting apparatus evinces an alliance with the plethodontid legion, because of the single compound fenestral plate and the pronounced isthmus fenestralis. Since the columella enters into the formation of the fenestral plate to a lesser degree than in the Necturidae, they must be considered an offshoot of the plethodontid stem, between the Necturidae and those still more specialized with regard to this feature. Furthermore, the greater reduction of the columellar element as a component of the fenestral plate in the Amphiumidae bespeaks a longer terrestrial period for this family than probably existed in the case of the Necturidae.

The Typhlomolgidae are unquestionably of the plethodontid legion, as indicated by the absence of lungs and by their similarity to Spelerpes larvae in most of their structural peculiarities, as pointed out by Emerson ('05) in her study of the anatomy of Typhlomolge. The sound-transmitting apparatus is decidedly of the plethodontid type, with a still smaller amount of the columella entering into the fenestral plate than in the Amphiumidae. Emerson expresses the opinion that the Typhlomolgidae should be included with the Plethodontidae. So far as the morphology of the sound-transmitting apparatus can be made out from the adult alone, it does not warrant such a union of families. A noticeable amount of the columella contributes to the plate part of the apparatus, which is not the case in any of the Plethodontidae. While not to be included with the Plethodontidae, Typhlomolge appears to have been derived in very close relations with them.

The Plethodontidae and Desmognathidae may be considered together, since the sound-transmitting apparatus in these families is identical. Here the columella is reduced to that extreme that it takes no part in the formation of the fenestral plate, merely becoming joined to it as the stylus columellae. In these families the identity of the columella is not lost, even after its attachment to the side of the plate. The differences between the sound-transmitting apparatus of the two families are slight, certainly of not more than generic value, which argues for the inclusion of the two groups in the same family, as was suggested by Moore ('00) in his study of the vertebrae and later adopted by Dunn ('17). Moore's point is well taken. The vertebrae of urodeles are acentrous. In such a shell of bone formed by the descent of the dorsal elements, a cavity necessarily results and its later partly or completely filled state, which resembles an opisthocoelian vertebra, is without significance in this connection.

As stated above, it seems clear that the type of sound-transmitting apparatus which obtains in the plethodontid legion could have come about originally only under the influence of or in adaptation to a terrestrial abode. For the existence of the isthmus fenestralis no reason suggests itself, unless it be that discussed in connection with Spelerpes in the first part of this paper or the persistence of the earlier cutting-out process of the operculum, which is here chiefly that of growth of the fenestral lips into the membrane.

The plethodontid legion, as a whole, has descended from terrestrial forms, the Necturidae, Amphiumidae, and Typhlomolgidae having returned to an aquatic abode. The extreme specialization of the sound-transmitting apparatus in these secondarily aquatic species leads to the belief that the legion has lived in a terrestrial environment from a very remote period. The unusually long larval period of Spelerpes and Gyrinophilus should be interpreted as a tendency of these modern species to return to an aquatic life as Necturus has done. The short larval period of Desmognathus, therefore, represents the normal period, or one in which the tendency is toward a more strictly ter-

restrial existence. The absence of a free larval period in Plethodon and others is to be looked upon as further progress toward a strictly terrestrial existence.

The relations and estates of the columella and operculum in urodeles, when coupled with other studies, lead to the conclusion that these animals, as a group, have not found favorable surroundings in the terrestrial zone. One after another has returned to the water permanently, and the relative duration of this secondary aquatic period is reflected in the structure and development of the animal. Others are now in the course of their regressive radiation, while a few, such as Plethodon, Autodax, and Hemidactylium, because of their small size and secretive habits, have succeeded in the terrestrial struggle and exhibit features of structure and life-cycle which show no regression, but rather an advance in adaptation to the dry zone.

SUMMARY

1. In all caudate amphibia two elements, columella and operculum, are present in the sound-transmitting apparatus.

2. In the most generalized state these elements exist independent of each other as in Amblystoma, Triton, Diemictylus, Siren, and Salamandra. The columella, being useful in aquatic life only, fuses in part with the ear capsule at transformation (Amblystomidae, Salamandra) or completely (Triton, Diemictylus, and probably Siren). The adult Cryptobranchidae, having failed to complete the metamorphosis, has the sound-transmitting apparatus in an arrested state of development, the columellar element alone being present.

3. In all of the other families (Necturidae, Amphiumidae, Typhlomolgidae, Plethodontidae, Desmognathidae) the representatives of the columella and operculum fuse to form a single plate. In this fusion there is, throughout the series of families, a gradual reduction of the columellar element from a state where it forms a portion of the fenestral plate, as in Necturus, to one where it becomes stylus only, as in Desmognathus.

4. The morphology of this apparatus shows the affinities and descent of the families as indicated in plate 6.

5. The nature and relations of the sound-transmitting apparatus indicate that these structures came into their present state in a terrestrial environment. Results gained from this study combined with others tend to confirm the view that the present-day aquatic species are secondarily so; that some species give evidence of just beginning a return to an aquatic abode; that others are still strictly terrestrial and likely to remain so.

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PLATE 1

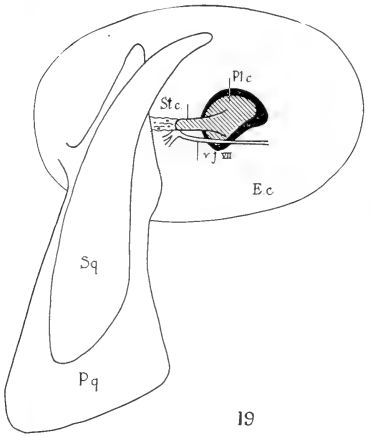
EXPLANATION OF FIGURES

A series of schemas to show the relations of the different morphological types of fenestral elements in caudate amphibians. The cross-hatched areas represent columella or portions derived outside the ear capsule. The elements derived from ear capsule are unshaded and represent operculum. Broken lines indicate a fusion of fenestral element and ear capsule.

19 Larval Amblystoma punctatum.
20 Adult Amblystoma punctatum.
21 Adult Diemictylus and Triton.
22 Adult Gyrinophilus representing the Plethodontidae.

ABBREVIATIONS

Ec., ear capsule
I.f., isthmus fenestralis
Op., operculum or functional fenestral plate of the adult
M.op., musculus opercularis
Pl.c., plate portion of columella
P.q., palatoquadrate
Sq., squamosum
St.c., stylus columellae
VII., nervus facialis
r.j.VII., ramus jugularis facialis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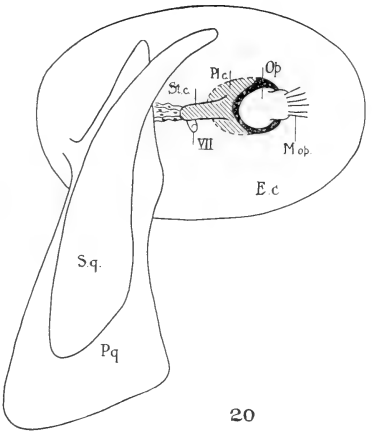
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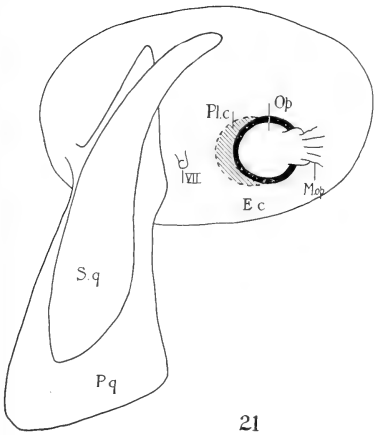
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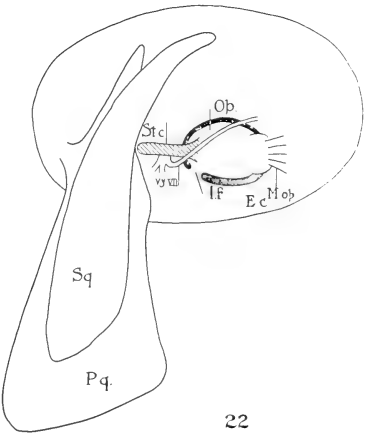
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22

PLATE 2

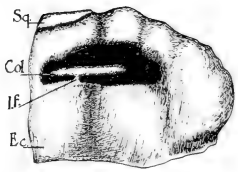
EXPLANATION OF FIGURES

Drawings of wax models of the ear capsule and sound-transmitting apparatus of Spelerpes bislineatus.

23 Larva 25 mm. long.
24 Larva 28 mm. long.
25 Larva 34 mm. long.
26 Mature larva 34 mm. long.

ABBREVIATIONS

Col., stylus columellae (columella)
C.S., level of text figure 5
D.a., dorsal arm of isthmus fenestralis growing into the fenestral membrane
E.c., ear capsule
I.f., isthmus fenestralis
Op., fenestral plate (operculum)
S., level of text figure 4
Sq., squamosum
Va., ventral arm of growing isthmus



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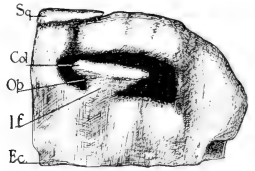

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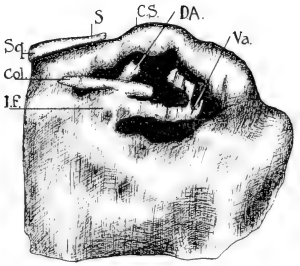

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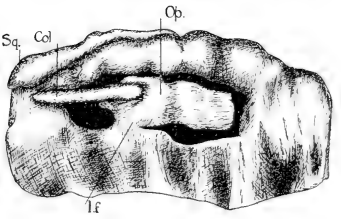

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PLATE 3

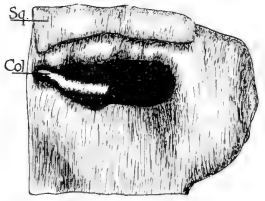
EXPLANATION OF FIGURES

Drawings from wax models of the ear capsule of Amphiuma means.

27 Embryo 30 mm. long.
28 Young adult 85 mm. long.
29 Adult 265 mm. long.

ABBREVIATIONS

Col., stylus columellae (columella)
I.f., isthmus fenestralis
Op., operculum (fenestral plate)
Ps., parasphenoid
Sq., squamosum
V.a., ventral arm of isthmus fenestralis



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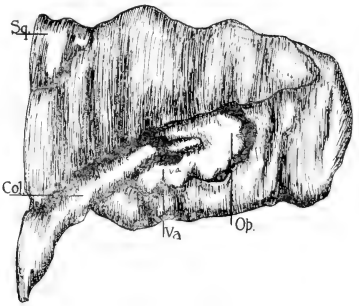

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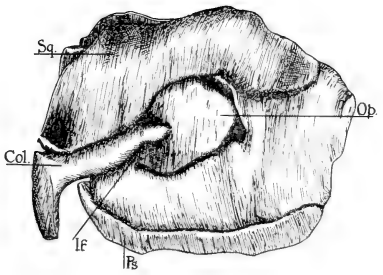

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PLATE 4

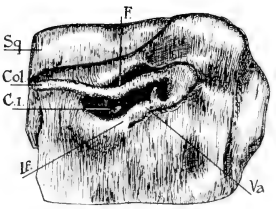
EXPLANATION OF FIGURES

Drawings of wax models of the ear capsule.

30 Larval Desmognathus fusca, 21 mm. long.
31 Larval Gyrinophilus porphyriticus 82 mm. long.
32 Adult Gyrinophilus porphyriticus.
33 Larval Necturus 48 mm. long.

ABBREVIATIONS

C.i., isolated area of cartilage formed in the fenestral membrane
Col., stylus columellae (columella)
Da., dorsal arm of invading isthmus fenestralis
F., level of the fusion of the stylus columellae and cartilage formed independently in the fenestral membrane
I.f., isthmus fenestralis
Op., fenestral plate (operculum)
Pl.c., portion of columella taking part in the formation of the fenestral plate
Ps., parasphenoid
Sq., squamosum
Va., ventral arm of isthmus fenestralis
VII, nervus facialis
r.j.VII, ramus jugularis facialis


F
Sq
Col
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Va

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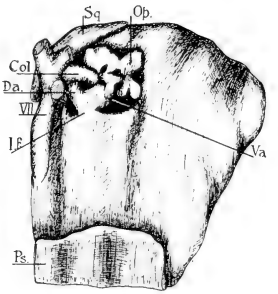
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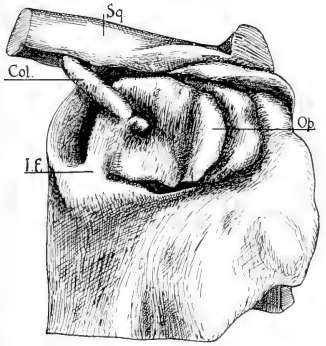
Ps

31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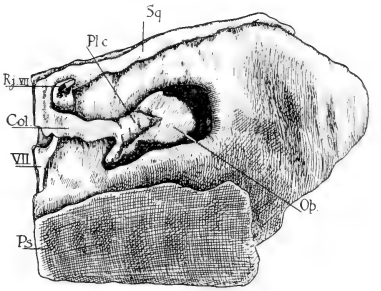

Sq
Pl.c
R.j.vii
Col
VII
Op.
Ps

33

PLATE 5

EXPLANATION OF FIGURES

Drawings from wax models of the ear capsule of Siren.

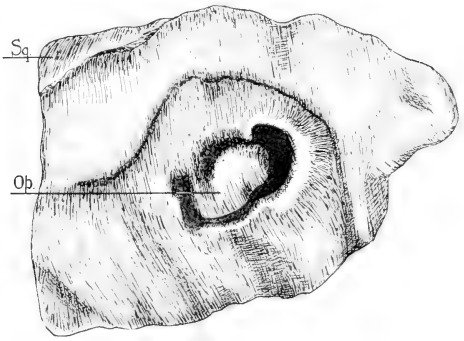
34 Specimen 133 mm. long.

35 Specimen 215 mm. long.

ABBREVIATIONS

Op., operculum, which in the younger specimen is not yet completely cut out.

Sq., squamosum.



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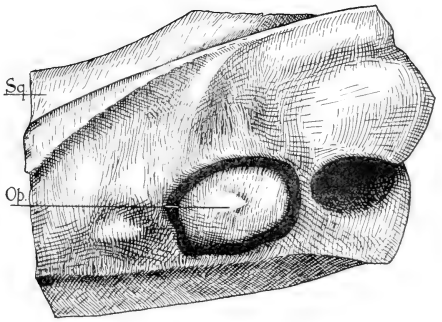

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PLATE 6

A phylogenetic arrangement of the several caudate families of amphibians suggested by the study of the sound-transmitting organs.

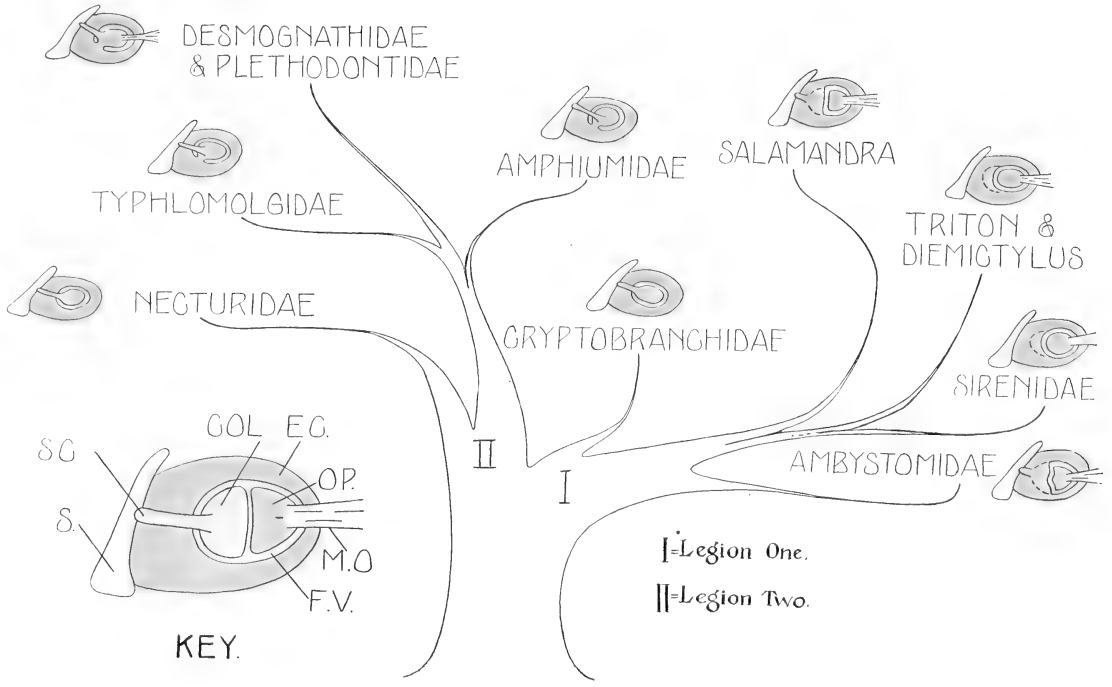
Red represents ear capsule and that portion of the fenestral plate (Operculum) derived directly from it.

Blue represents that portion of the sound-transmitting apparatus which is extraotic in origin (columella).

Broken lines represent a fusion of parts.

ABBREVIATIONS

Col., columella
E.c., ear capsule
F.v., fenestra vestibuli
Mo., musculus opercularis
Op., operculum
S., suspensorium of the lower jaw
S.c., stylus columellae


DESMOGNATHIDAE
& PLETHODONTIDAE
TYPHLOMOLGIDAE
NECTURIDAE
AMPHIUMIDAE
SALAMANDRA
TRITON &
DIEMICTYLUS
GRYPTOBRANCHIDAE
SIRENIDAE
AMBYSTOMIDAE
II
I
I=Legion One.
II=Legion Two.
SC
GOL
E.C.
O.P.
S.
M.O
F.V.
KEY.

Resumen por el autor, G. W. Tannreuther.
Universidad de Missouri.

El desarrollo de Asplanchnia ebbesborni (Rotíferos).

La formación y segmentación de los óvulos puede seguirse paso a paso dentro del animal vivo a causa de su transparencia. La segmentación, en muchos puntos, tales como la dirección, orden y marcha de la misma, presenta el mismo caracter que la de los anélidos, formándose tres generaciones de ectómeros. La región ventral de los ectómeros corresponde al futuro extremo anterior, mientras que la que ocupan los macrómeros, A, B y C corresponde mas al extremo posterior. Durante la gastrulación el macrómero grande 3D pasa al centro del embrión. A, B y C permanecen en la superficie del extremo posterior del embrión y sus derivados están relacionados mas directamente con la producción del voluminoso pié embrionario. 3D (E) origina el sistema reproductor con unas cuántas fibras musculares y todo el sistema digestivo, con la excepción del stomodaeum. Los derivados de los ectómeros producen las demás estructuras. Todos los órganos, (con excepción del sistema digestivo del macho) son funcionales y están bien desarrollados en ambos sexos. El animal produce óvulos machos, hembras y otros en estado de reposo. Todos los óvulos, excepto los en reposo, son transparentes; los últimos poseen abundante vitelo. Los machos y las hembras no son originados nunca por el mismo animal, mientras que los embriones machos y óvulos en reposo son producidos por la misma hembra. Los óvulos machos y los ultimamente mencionados difieren en estructura y solamente los en reposo son susceptibles de fecundación. La naturaleza y marcha del desarrollo indican que los rotíferos no son larvas trocóforos de los anélidos, que han persistido, sino mas bien que los rotíferos y anélidos se han originado a expensas de una misma forma ancestral, habiendo alcanzado los anélidos un estado mas elevado en la historia de su evolución.

Translation by José F. Nonidez
Carnegie Institution of Washington

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THE DEVELOPMENT OF ASPLANCHNA EBBESBORNII (ROTIFER)

GEORGE W. TANNREUTHER

Zoological Laboratory, University of Missouri

TWENTY-ONE TEXT FIGURES AND SEVEN PLATES

CONTENTS

INTRODUCTION

The size of the adult rotifer, in many instances, has made the study of development a difficult problem. A great descriptive mass of literature has grown up around this group of animals, but with the exception of a very few detailed accounts of their structure, the writers have contented themselves with the description of the external form.

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The morphology of the Rotatorian family Flosculariidae was worked out by T. H. Montgomery ('03) in considerable detail. Zelinka ('92) published an account of the development of Callidina ruseola. Jennings ('96) traced the cell lineage of Asplanchna herricki, for a few generations, but did not correlate the cells of the early embryo with the adult structures.

Some rotifers are very transparent; this makes possible, not only a detailed study of the position and relation of the various structures, but an investigation of the origin, growth, and cleavage of the eggs. Where complete development occurs within the parent, a sequence of the different stages from the first appearance of the egg to the time of birth can be followed with a considerable degree of accuracy. The extreme transparency of the adult Asplanchna ebbesbornii, makes possible a detailed study of the successive stages in development.

The more important points in development may be summarized as follows:

1. The cleavage is unequal and regular. A small cleavage cavity is present. The gastrula is formed by a modified epiboly. The blastopore occurs at the posterior end of the developing embryo.

2. The first cleavage plane is at right angles to the future median longitudinal axis of the adult. It divides the egg into two very unequal cells. The smaller cell is ectodermal and mesodermal, while the larger cell contributes to the three germ layers.

3. The gastrula consists of an outer layer of epithelial cells enclosing an inner cell mass. The outer layer includes all of the derivatives of A, B, and C and the three cells d^1, d^2, and d^3 derived from D. The inner cell mass includes all of the remaining cells.

4. The outer layer or ectoderm gives rise to the cuticle, hypodermis, brain, excretory system, trochal disc, cilia, buccal pouch, and musculature. The inner cell mass derived from 3D produces the remaining parts of the digestive system and glands, the reproductive system, and a few of the muscles which control it.

5. The origin of the various organs cannot be traced back to any definite cell or cells of the early cleavage stages, but can be directly associated with definite regions of the early gastrula. It is true, however, that the products of any one cell A, B, C, or D, in the production of the ectoderm, can be definitely followed and localized in the gastrular ectoderm, but there are no structural differences to mark off these regions. The ectodermal cells of any region possess the same potentiality in producing muscle fibers, regardless of their origin. It is impossible to tell definitely the differentiation of the digestive and reproductive systems until late cleavage.

6. The derivative of the blastomere B form the ventral, those of D the dorsal, those of A the left lateral, and those of C the right lateral ectoderm of the adult animal.

7. The brain on the median dorsal side is derived from the ectoderm at the anterior end. The urogenital sinus is formed by a solid ingrowth of the ectoderm on the median dorsal side at the posterior end above the base of the embryonic foot. Its position is considered as being ventral in the adult, due largely to the disappearance of the foot in later embryonic life. The embryo in its early development is curved ventrally, making the dorsal side appear quite long in comparison to that of the ventral. The mouth is ventro-anterior.

NATURAL HISTORY

The rotifer Asplanchna ebbesbornii is not very abundant in this immediate locality. The material was collected from small rain pools and placed in aquaria, filled with tap-water. This particular rotifer was first observed in one of the freshly prepared cultures in January, 1916. They persisted about two weeks and disappeared. They reappeared in the following March and continued for two weeks. The rotifers appeared again in the following May. In each instance about two months elapsed between the times of their appearance. This periodicity continued until December, 1918, when this paper was completed. In each cycle of appearance males, females, and resting eggs were formed in about the same proportions. The cultures were kept indoors at laboratory temperature.

The January and March cycles were not studied very extensively. The studies proper were begun with the May cycle, 1916, in tracing out the cell lineage. The adults are extremely transparent, and by holding them in any desired position under a supported cover-slip the cleavage of the individual cells can be traced step by step. Two distinct kinds of adult females exist, which are structurally similar as far as can be determined under a magnification of 100 to 150 diameters. The one reproduces females parthenogenetically, and the other males parthenogenetically or resting eggs, which carried the cycle of one period to their next reappearance. The resting eggs pass through their early stages of cleavage before deposition. There are, however, two kinds of resting eggs: a very thin-shelled one, with a single shell membrane, and a thick-shelled egg, with a double shell membrane. The thin-shelled egg develops with the same rapidity as the parthenogenetically produced individuals and hatches out immediately after deposition. Two polar bodies are formed in each kind of resting egg, which do not develop unless fertilized.

Females and resting eggs or females and males are never produced by the same individual. The parthenogenetically produced males and females are sexually mature at birth. The uterus of the young females often contains embryos in the late cleavage stages at the time of their birth. The two kinds are practically the same size at birth. Copulation occurs almost immediately after the birth of the males. The male may copulate with either kind of female. The uterus and the oviduct of the parthenogenetic producing female often contain sperm, but neither males nor resting eggs are produced by this particular individual. On the other hand, the uterus and oviduct of the male-producing parent may contain an abundance of sperm, and yet produce males only, or again, they may produce both males and resting eggs. There is no definite sequence in the production of males and resting eggs by the same parent. A single male may be produced and all the remaining become resting eggs, or vice versa. Or, on the other hand, it is not unusual to find the two alternating. The following is a good example: the sequence

was as follows: two resting eggs, one male, one resting egg, one male, one resting egg, one male.

In case of the female which produces the resting eggs the vitellarium undergoes a marked change after impregnation. The yolk spherules become larger and more abundant and give the vitellarium a very dark color. The yolk is first produced at the point where the oviduct takes its origin. This process continues until the vitellarium is completely filled with yolk. Where males and resting eggs are produced by the same parent, the yolk is produced at intervals just before the resting egg begins its growth in the ovary. The male eggs are free from the dark yolk and remain transparent. Impregnation has no effect on the vitellarium of the female-producing individual in the production of yolk. Figures 7 to 11 show the single thin-shelled and the double thick-shelled resting eggs. In figure 12 the spermatozoa are shown in different stages of development.

In many instances the sperm of the sexually mature male (before birth) would escape from the testis and become deposited in the uterus of the parent and bring about the production of resting eggs. In other cases the male was little more than a large sperm sac. The male embryos developed normally until the early differentiation of the reproductive organs. At this stage of development all of the cleavage cells, except those directly concerned in the production of the sperm, ceased dividing, took on a vesicular appearance, gradually deteriorated, and functioned as food for the developing sperm. The sperm, when mature, escaped through the egg membrane into the lumen of the uterus. This condition accounts, to some extent, for the few free-swimming males in the cultures.

The males are structurally degenerate at birth. The digestive tract is very rudimentary (figs. 5 and 6) and never opens to the exterior. The males are very short-lived, and few free-swimming individuals are present at any one time. The males do not increase in size after birth (figs. 5 and 6). On the other hand, the females increase to at least four to six times their size at the time of birth (fig. 1). The embryology of the male and the female developing individual is practically the same throughout

the different stages of development. One polar body is formed in the female egg, while in all others two polar bodies are formed.

The individuals hatching from the resting eggs are always parthenogenetic females. But the next generation is of two kinds, one producing females parthenogenetically and the other producing males parthenogenetically or resting eggs.

MATERIAL AND METHODS

The rotifers were removed from the different cultures with a pipette and placed in watch crystals. The excess water was then removed and the fixing fluid added. Bouin's fluid gave the best results. The animals were preserved in 70 per cent alcohol until used. If allowed to stand indefinitely in alcohol the individuals turn brown and are not very satisfactory for study. For whole amounts Delafield's haematoxylin and erythrosin were used. Either stain gave good results. The changes from alcohol to xylol and from xylol to the mounting medium must be made very gradually. A few drops of carbo-xylol and clove oil will help considerably in the process. The cuticle is very resistant and will cause considerable shrinkage and distortion if the change be made too quickly. The same caution must be taken in clearing specimens that are to be imbedded and sectioned. Iron-alum haematoxylin and erythrosin gave the best results for sections. In the case of whole mounts, the coverslips should be supported.

ORIGIN AND FORMATION OF OVUM

The reproductive organs are composed of the vitellarium, ovary proper, oviduct, and uterus. The uterus opens into the urogenital sinus. The vitellarium is somewhat U-shaped (fig. 4), very transparent when free from yolk, and contains many large nuclei. The ovary (figs. 1 to 4) occupies a very small area at the base of the vitellarium.

Parthenogenetic ova: The ova are very small, and a single ovum at regular intervals begins its growth. The contents of the growing egg is derived directly from the vitellarium. The

passage of cytoplasmic and yolk granules into the egg is visible under a low power. When the egg reaches the end of its growth period, it is separated from the ovary (fig. 4), and enters the upper end of the oviduct, which, in reality, encloses the greater part of the ovary. A single polar body is formed. In most cases immediately after this maturation, the following egg begins its growth. Two eggs may begin their growth at the same time, but this is very unusual. In the case of the male-producing eggs, two polar bodies are formed, the first of which often divides. The origin, formation, size, and development of the female and the male-producing eggs are identical (figs. 2 to 4).

Sexual or resting ova: The origin and growth of the resting eggs are similar to that of the parthenogenetic eggs. The vitellarium, however, is very dark from the presence of a rich supply of yolk. The yolk passes directly from the vitellarium into the growing egg. Immediately after maturation and fertilization, a very thick inner shell is formed from the cytoplasm. The contents of the double-shelled egg cannot be studied except in sections In many of these resting eggs no inner shell membrane is formed. They are about the same size as the thick-shelled eggs, but are more transparent and contain less yolk. Their cleavage stages can be followed without the aid of sections. The number of resting eggs in the uterus at any one time varies from one to eight. In case of the female or male-producing parent, there may be as many as sixteen embryos in the oviduct and uterus at the same time, ranging from the early cleavage stages to the mature young (fig. 2).

CLEAVAGE

1. Designation of the cleavage cells

The nomenclature adopted in the designation of the cleavage cells is a modification of the system used by previous investigators on cell lineage. The first four cells (macromeres) are designated by the capital letters A, B, C, and D. The generations of micromeres (ectomeres) by the small letters a, b, c, and d. The first index number indicates the generation to which the ectomere

belongs. Thus a^{1}, $b^{1.2}$, $c^{1.1.2}$, or $d^{1.1.1}$, all belong to the first generation; c^{2}, $b^{2.1}$, or $d^{2.3}$, belong to the second generation, and $a^{3.1}$, $b^{3.2.2}$, $c^{3.2}$, or $d^{3.3}$, etc., belong to the third generation.

On account of the peculiar shifting of the macromeres A, B, C, and D in the formation of the first quartette, A, B, and C take a position more anterior and D posterior, instead of at the vegetal pole, as in annelids. When a cell divides, the product receives the designation of the parent cell with the addition of a further index number; thus, $c^{2}\begin{cases} c^{2.1} \\ c^{2.2} \end{cases}$. The cell D, after the formation of d^{2} and d^{3}, is designated by the capital letter E; it gives rise to the reproductive system and all of the endoderm (digestive system) except the stomodaeum and the pharynx.

2. Nature of cleavage

First cleavage: Immediately after maturation the nucleus passes from the surface toward the center of the egg, but nearer the anterior end. The first cleavage spindle is formed about thirty minutes after maturation (figs. 13 to 16), the time varying somewhat with external conditions. Low temperature retards the rate of cleavage. The first cleavage spindle occurs in the plane of the long axis of the egg (fig. 7). It passes through the region of the polar body or bodies and divides the egg into two very unequal parts, AB and CD (figs. 15 to 17). The smaller cell, AB, is anterior and the larger cell, CD, is posterior. The cleavage furrow at first is deep and the cells are rounded, but before the second cleavage occurs the cells flatten at their point of contact and the egg becomes more elliptical with the first cleavage plane scarcely visible.

The granular content of the cells is uniform, with very few yolk bodies visible. The region immediately surrounding the nucleus is almost free from cytoplasmic granules and makes it possible to follow the nuclear activities in the process of division in the living egg. The first cleavage in the female- and the male-producing egg is the same. It occurs at right angles to the future longitudinal axis of the adult. The second cleavage plane occurs

at an angle of about 45° with that of the first. The two cells divide at different times. These two cleavages combined correspond to the second cleavage as it occurs in many of the annelids and molluscs. The cell CD divides first into two very unequal parts (figs. 18 and 19). The division of AB is nearly equal (figs. 18 to 20). Shortly after the second cleavage, a slight shifting occurs as the cells flatten (figs. 19 to 24). The largest cell, D, is posterior, B median anterior, C right, and B left (fig. 21), with reference to the median axis of the future animal. The largest cell, D, always divides first in the formation of the quartettes. The orientation of the four-celled embryo is very simple and agrees with that of the annelids at the same stage of development. From this point forward, however, the position taken by the resultant cleavage cells is no longer comparable with that of the annelids, but the sequence of cleavages in the following stages is very similar.

Third cleavage: In the formation of the first generation of ectomeres (d^1, b^1, c^1, a^1), the cell D divides first, the new cell is budded off in a dorsal anterior direction on the median dorsal side of A, B, and C (text fig. b and figs. 23 and 24), making a five-cell stage. In this process the polar body is carried forward to the anterior end with the cell d^1. While the cleavage spindle is forming for the production of the micromere d^1, the cells A, B, and C elongate in an anteroposterior direction (text fig. c and figs. 24 and 25), so that the cleavage plane of A, B, or C is not horizontal, but in a dorsoventral direction at right angles to the long axis of the embryo (figs. 26 to 31). Thus, instead of having the micromeres above the macromeres as in many forms, the cells a^1, b^1, c^1, and d^1 are on the same level with A, B, and C (figs. 31 and 32). The division of the macromeres A, B, and C is nearly equal in the formation of the first generation of ectomeres. They do not divide simultaneously, but in the invariable order C, B, A. Thus there occurs successively a six-, seven-, and eight-cell stage (figs. 30 to 32). In the formation of the eight-cell stage B and b^1 are pressed ventrally by d^1 (figs. 24 and 31). In the eight-cell stage d^1 is median dorsal, B and b^1 median ventral, C and c^1 right, A and a^1 left, and D posterior extending

dorsoventral (figs. 31 to 33). The micromeres form the anterior end. In a few instances, C, B, and A divided in a nearly horizontal plane, thus placing the micromeres above the parent cells instead of on the same level with them (text figs. d and e and fig. 33). This mode of division, however, is very unusual, but is comparable with that of the annelids and polyclades.

At the completion of the eight-cell stage, the embryo often assumes the shape of the one-cell condition and the cleavage furrows are scarcely distinguishable. The embryo at the different stages of development is very plastic and may asssume almost any shape under abnormal pressure. If the egg be removed from the reproductive organs, with the egg membrane intact, after cleavage has begun, normal development will continue. By slight pressure the individual cells of the eight-cell stage can be separated. The isolated cells seldom continue to divide, but begin to deteriorate almost immediately.

Fourth cleavage (sixteen-cell stage): A nine-cell stage is reached by the formation of d^2 from the large cell D on the median dorsal side, in an anterior direction (figs. 34 and 35). As d^2 is formed, d^1 is carried around the dorso-anterior end. Next, d^1 divides in an anteroposterior direction (fig. 36). Following this, the macromeres A, B, and C and their micromeres divide in an anteroposterior direction. The division occurs in the order C and c^1, B and b^1, A and a^1, thus producing a twelve-, fourteen-, and sixteen-cell stage, respectively (figs. 34 to 38). The embryo is now composed of four rows of cells with four cells in each row (figs. 38 to 44).

The fifth cleavage: The derivatives of D divide in the following order: d^2, d^1, $d^{1.1}$, thus producing a seventeen-, an eighteen-, and a nineteen-cell stage (figs. 45 to 48). The cleavage spindles in the C, B, and A rows indicate the direction of the cleavages in passing from the nineteen- to the thirty-one-cell stage (figs. 45 to 48). In a few of the embryos, as in annelids, D budded off a third cell, d^3 (figs. 46, 47, 49, and 50). This extra cell, when produced, has no special significance in the future development of the individuals which bear it; d^3 and its derivatives will not be considered in the further description of the cleavage stages.

At the close of the fifth cleavage, the quadrants A, B, and C are composed of two rows with four cells each, and the quadrant D of two rows with three cells each, including the large entoderm cell D, making thirty-one cells in all. The comparative sizes of the cells are shown in the different figures. During the fifth cleavage the cells withdraw toward the exterior (fig. 55) and

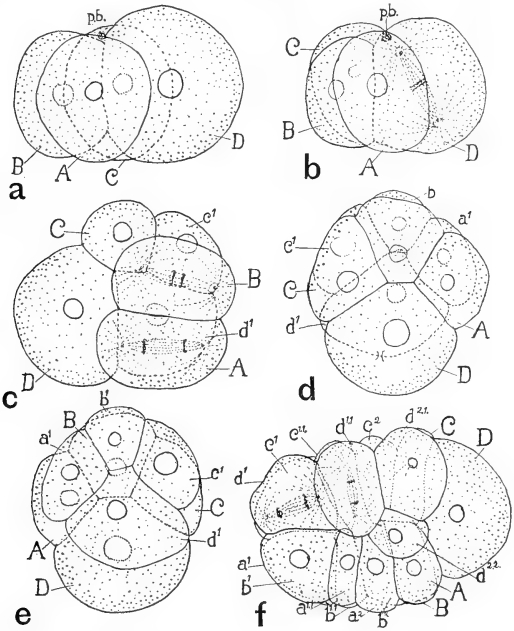


Fig. a Four-cell stage, left side.

Fig. b Four-cell stage, left side, showing the cleavage spindle in the formation of the ectomere d^1.

Fig. c Six-cell stage, ventral side; shows the anteroposterior extension of cells.

Figs. d and e Eight-cell stage, ventral and dorsal sides; shows the position of the first quartette of ectomeres similar to that of annelids. This condition is unusual.

Fig. f Seventeen-cell stage, left side.

form a cavity, which later is occupied by the large cell D. Before the fifth cleavage is complete, the anterior end of the cell D is partially covered by the cleavage cells immediately in front of it (figs. 45 to 50).

Sixth cleavage: After the formation of the small cell d^4, the cell $d^{1.1.1}$ (fig. 51) divides. Next a small cell d^5 is formed from D. The first cell of the sixth cleavage to divide is $d^{2.1}$, etc. The sequence of cell formation in the sixth cleavage is similar to that of the fifth, the derivatives of the D quadrant dividing first, then those of C, B, and A. The cell lineage of any one quadrant can be followed indefinitely. Figure 51 represents the beginning of the sixth cleavage. From this stage the surface cells will not be labeled, as it does not contribute to the understanding of the further development. The sixth cleavage doubles the number of cleavage cells on the surface. It does not increase the number of rows in each quadrant, but the number of cells in each row is doubled. The embryo at the end of the sixth cleavage is composed of the following cells: the D quadrant contains two rows of six cells each, and d^4 and d^5. The C, B, and A quadrants each contain two rows of eight cells each. With the large entoderm cell D, there are thus sixty-three cells in all.

3. Gastrulation

Gastrulation, which begins during the close of the fifth cleavage, adheres more strictly to the epibolic type. Immediately after the formation of d^2, the surrounding cells at the anterior end of D begin to extend over its surface (fig. 48). The embryo when viewed from the posterior end (figs. 52 and 53) shows the position of the surface cells with reference to D. The spindle indicates the direction in which d^4 is formed. Figures 52 and 54, later stages during the sixth cleavage, show the method of overgrowth on the surface of D (E). Gastrulation is a double process; while the surface cells are extending posteriorly over the surface of E, the large cell itself is migrating into the interior, a result of the pressure of the surrounding cells, and the cavity within the embryo, which began its formation before the sixth cleavage started.

During gastrulation the embryo shortens and increases in width, as shown in figures 55 to 57. The formation of the central cavity (figs. 57 and 58) and gastrulation occurs very rapidly. The entire process requires about fifty minutes and can be demonstrated in the living egg. Figures 58 and 59, a sixty-four-cell stage, viewed from the dorsal and ventral sides, respectively, show the embryo at the end of gastrulation. The blastopore, situated at the macromere end of the embryo, is rather large at first and is surrounded by eight cells, two belonging to each of the four quadrants (figs. 52 to 54).

Gastrulation brings about a fundamental change in the relation of E to the remaining cells of the embryo. At first it formed the posterior end of the cleavage cells, but at the end of gastrulation it occupies the central region of the growing embryo and is completely enclosed by the surrounding cells (figs. 58 and 59). The large cell E is now designated as the mesentoblast, and, on account of its new position is destined to assume a new rôle in the development of the embryo. The embryo at the end of gastrulation contains about 200 cells, which are divided into two distinct regions; the mesentoblast including d^4 and d^5, and the epithelial ectoderm, which, at the anterior end, shows the beginning of a double layer (figs. 58 and 59).

SEGREGATION OF THE GERM LAYERS

In Asplanchna ebbesbornii a distinct segregation of the germ-layers begins with the formation of the cell d^2 or d^3, the nine- or seventeen-cell stage. The cell d^3 is not usually formed, hence the variation in the number of cells at the time of segregation. The large posterior cell D is destined to become entomesodermal, and all of the remaining cells are ectomesodermal.

1. Ectoderm

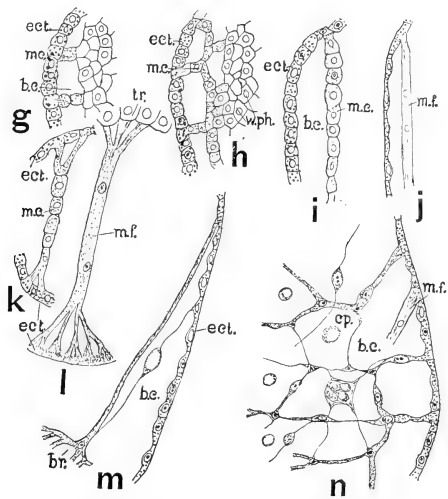
The dorsal part of the ectoderm is derived from the quadrant D, the right, the left, and the ventral parts from C, A, and B, respectively. The three cells of the quadrant D, d^1, d^2, and $d^{1.1}$, are the first to divide in the formation of the ectoderm during

the fifth cleavage. Each cell divides unequally. The anterior cell divides vertically, while the other two divide transversely and parallel to the long axis of the body (fig. 48). For further account of the cleavage process in the formation of the early ectoderm, see description given for the later cleavage stages. Figures 55 to 57 represent the earlier stages in which the cleavage cells become arranged into a definite epithelial layer. The cavity shown in these figures is in reality the early body cavity, since the epithelium later becomes the definitive body wall. A later condition of the ectoderm is shown in figures 67 and 68. From this point onward the division of the epithelial cells is very irregular and their products become differentiated into the definitive ectoderm and its various derivatives. At first the definitive ectoderm is represented by an epithelial layer composed of large nucleated cells, and is in immediate contact with the organs forming within (figs. 77 to 90). But as the different organs reach their complete development, the body cavity becomes more and more marked and the cells of the ectoderm are drawn out into a very thin epithelial layer, with the boundary of the cells no longer visible (figs. 96 to 104). The cuticle is a very thin layer formed by a secretion from the ectoderm shortly before birth. The cuticle is usually free from markings and has a smooth surface. Figure 104 represents the condition of these various structures at the time of birth.

2. Mesoderm

In the late gastrula stage (figs. 63 to 70), the outer epithelial layer of the embryo at different regions begins the proliferation of cells on the inner surface. These cells contribute directly to the formation of the mesoderm, which later becomes differentiated into the muscular system. It is impossible to distinguish in the embryonic ectoderm the cells which directly give rise to the mesoderm. Apparently all parts of the ectoderm possess the same potentiality in the process. These cells, in their formation, remain connected with their point of origin. Later these proliferated cells show an apparent connection or fusion with the

inner cell content (the invaginated stomodaeum and the derivatives of the mesentoblast) (text figs. g, h, and q). Other rows of proliferated cells at either end are connected with the ectoderm (text fig. k). Many of these cell processes have several attachments (text fig. h), but in their further development some of these points of fusion are lost and the developing muscle remains attached to the later formations, which they are destined to control. The muscle fibers, in their early formation, are com-



Figs. g and h Early muscle cells connecting ectoderm with inner structures.

Figs. i and j Early and definitive stages of muscle-fiber development.

Figs. k and l Shows method of attachment in the early and late development of muscle fibers.

Fig. m A completely formed muscle, showing attachment to brain and ectoderm.

Fig. n A network of muscle fibers extending across body cavity.

ABBREVIATIONS

b.c., body cavity
br., brain
cp., corpuscle
ect., ectoderm
m.c., muscle cell
m.f., muscle fiber
tr., trochus
w.ph., wall of pharynx

posed of cellular processes (text fig. k), which, later, as development progresses, lose all traces of cell boundaries, with a few nuclei persisting (text figs. i to m). The method of attachment in one of the completely formed muscles is represented in text figure l. It is attached anteriorly to the corona and at its opposite end it is anchored to the ectoderm. Some of these muscle fibers show a distinct cross or longitudinal striation with few nuclei. In the mature embryo (text fig. n), many of the muscle fibers are drawn out and form a delicate network, which extends through the different regions of the body cavity. Some of these fibers are mere lines and are hard to distinguish. Many of the apparent migratory cells within the fluid of the body cavity are directly connected with very delicate processes (text figs. m and n), while others are mere floating corpuscles, which are highly vacuolated. The various structures within the body, as well as the trochal disc, are kept in constant motion by the activities of the various muscle fibers. No attempt was made to represent the position of the different muscles in the various figures drawn.

3. Entoderm

During the early part of the seventh cleavage, the blastopore becomes completely closed (figs. 59 and 60). The cells designated as the entoderm include the large cell E and the two small cells d^4 and d^5, which are formed from E during the sixth and seventh cleavages of the embryo. Figures 58 to 60 show the first stages in the cleavage of E, which corresponds to the eighth cleavage. This cleavage is unequal and separates a smaller cell, E^1, from a larger cell, E^2, posteriorly. Figure 60 is a dorsal view of the embryo and shows the condition of the ectoderm and entoderm at the beginning of the eighth cleavage. The ectodermal cells during the ninth cleavage divide very rapidly and are difficult to follow. Immediately after the first cleavage of E, the cell E^2 divides equally and transversely, or at right angles to its first cleavage. Figures 61 to 64 show the different stages in the process of division. The embryo, as a whole, is very plastic and becomes more spherical during the cleavage of

E^2 (fig. 62). Figure 63 represents a 250-cell stage, and by turning the embryo in different positions all of the cells can be recognized, but it is impossible to tell the exact boundary of the cells derived from any one quadrant. The derivatives of D always take the initiative in division. Figure 63 represents the eighth cleavage complete and the beginning of the ninth. In figure 64 the entodermal cells do not completely fill the central cavity of the embryo. Next, the anterior entodermal cell E^1 divides very unequally and forms $E^{1.1}$ and $e^{1.2}$ (fig. 65). The smaller cell, after a few divisions, is difficult to follow. Immediately after the division of the anterior cell E^1, the cells $E^{2.1}$ and $E^{2.2}$ divide equally. The spindles of these divisions are shown in figure 65. The division is equal and takes place in an anteroposterior direction. The entoderm is now composed of five large cells and three smaller ones.

Figure 66 represents an embryo with the five large entodermal cells viewed from the left side. Spindles are present in each of the cells for the following cleavage. Figure 67 represents an optical section a little earlier than the preceding, viewed from the right side. The ectodermal cells are somewhat contracted and do not fill the central cavity. The cells of the embryo at the dorsal posterior end multiply very rapidly, extend backward over the blastopore, and, in a later stage, contribute to the formation of the temporary foot. At the next division each of the five entodermal cells divides dorsoventrally and forms two layers of five cells each, as shown in an optical section in figure 68, with the ectoderm removed. Figure 69 shows the same stage with the ectoderm intact. At the next division each of the five upper and the lower entodermal cells divide equally. The cleavage occurs in an anteroposterior direction and produces twenty large entodermal cells, as shown in figure 70, an upper view. From this point forward no attempt was made to follow the individual cleavage cells. Figure 71, a ten-hour embryo, shows about the same stage as the preceding from the right side. The position of the entoderm is indicated by a dotted outline. The beginning of the foot and the first stage in the formation of the stomodaeum is evident from the ventral side. Figure 72, a

little later stage than the preceding, from the right side, shows the position of the central entodermal mass and its relation to the embryo as a whole. The ectodermal cells at the anterior end of the embryo now divide very rapidly and later contribute in part to the formation of the stomodaeum and the pharynx (figs. 73 to 75). The central mesentodermal mass of cells becomes differentiated into two distinct regions (figs. 76 and 77), the entoderm proper, which produces the stomach, oesophagus, and digestive glands; and the part which produces the reproductive organs with a few of its controlling muscle fibers.

FORMATION OF THE TROCHAL DISC

The developing embryo is divided into three distinct regions; the body, the head bearing the trochal disc, and the foot. The foot is an embryonic structure and is absorbed before the birth of the individual.

The trochal disc begins as ectodermal prominences or growths, due to a proliferation of cells on the ventro-anterior region. Figure 73, viewed from the ventral side, shows a small lateral fold on either side. A stage little later than the preceding is shown in figure 74 from the right side. These ectodermal folds extend toward the posterior end, but only the anterior ends contribute to the trochal disc. This figure shows the first steps in the differentiation of the animal into three distinct regions (head, body, and foot). The folds are more developed in figure 78, and the large central depression between the lateral folds is open at either end. Later stages are represented in figures 81 and 83. The position of the mouth is visible in figure 81. The embryo at this stage of development is considerably curved and the ectodermal folds posterior to the stomodaeum have begun their development. The extreme anterior folds later become the dorsal part of the trochal disc. The position of the mouth is now more definite. The lateral, anterior, and posterior folds are now continuous (fig. 89). This figure shows the mouth close to the posterior prominence, which, later, when the embryo straightens out, becomes ventro-anterior. Figures 91 to 93 show the foot at its maximum development, with its two pointed toes.

It projects forward over the anterior end of the embryo and obscures a part of the fold which contributes to the formation of the disc. These figures show how the disc is depressed posteriorly on the ventral side. The dorsal part of the disc is indicated in figure 92. The entire disc in its final stage of development with the outer row of cilia, is represented in figure 99, ventral view. The trochal disc is very retractile, and its activities are regulated by a number of well-developed muscle fibers. The cilia extend into the buccal pouch, but not into the pharynx. The ciliary wreathes serve for locomotion and feeding.

DIGESTIVE SYSTEM

The major portion of the digestive system is derived from the large mesentoblast cell E, the stomodaeum and pharynx from the ectoderm. The early stages in the formation of the anterior end of the digestive tract begin as an invagination on the ventro-anterior end (figs. 71 to 76). The development of the head region in the formation of the trochal disc is closely associated with the origin and growth of the anterior end of the digestive tract, and the prominence of the trochal folds is accentuated by the stomodeal invagination. Figures 76 and 77 represent optical sections from transparent whole mounts in the early stages of differentiation in the region from which the pharynx develops. The embryos are folded ventrally, making the dorsal side appear abnormally long. These figures show that two distinct regions are recognizable—an outer epithelial portion, which gives rise to the definitive ectoderm and the musculature, and an inner slightly differentiated region, which produces part of the enteric canal and the entire reproductive system with a few muscle fibers directly connected with it. As the cells invaginate to form the pharynx, the products of the mesentoblast are forced more and more posteriorly (figs. 75 to 77). The cells on the posterior face of the invaginated cavity become several layers thick and form the ventral and posterior walls of the pharynx, from which the jaws are later developed. The anterior and dorsal walls of the pharynx seldom become more than one layer of cells in thickness. Rudimentary salivary glands are often present in connection with the walls of the pharynx (fig. 102).

The inner cell mass, as shown in figure 77, becomes differentiated into the parts which produce the pharynx, the oesophagus, stomach, and the gastric glands, and those which form the reproductive structures. A later differentiation is indicated in figure 79, where the lumen of the stomach is present and the pharyngeal wall has become several layers thick on its posterior face. Figures 80, 82 and 88 represent horizontal sections of the preceding figure at different levels. The space which corresponds to the body cavity is evident at different points. These three sections as a whole show little differentiation and are rather hard to interpret unless directly compared with the figure from which they were taken. In the further growth and curvature of the embryo the upper wall of the pharynx fuses with the entodermal cells (figs. 85 to 87) forming the region which later is differentiated into the oesophagus and the gastric glands. In a medium longitudinal section of an embryo (fig. 87) corresponding to figure 86, a large concavity is evident on the ventral side. This is true of all embryos, when the invagination in the formation of the pharynx has reached its maximum extent in a dorsoposterior direction. Figure 89 (ventral view) represents the condition of the embryo, when the pharynx has reached its maximum development. The anterior and posterior ends of the embryo are in immediate contact and the mouth is in the center of the embryo when taken as a whole. An optical section of the same stage (fig. 90) shows the position of the mesentodermal cells as they are forced nearer the posterior end. This shifting is due to the invagination of the ectoderm in the formation of the anterior end of the enteric canal. The above figure shows the connection of the pharyngeal and the entodermal cells.

The embryo at this stage of development has reached its maximum curvature and growth in length (figs. 91 to 93), but does not resemble the adult rotifer. The embryo now begins to straighten out (figs. 94 and 95), the foot is gradually absorbed, and the mouth is carried more anteriorly (fig. 96) during the process. The invaginated cavity is differentiated into the buccal cavity and the pharynx proper. The embryo during its period of maximum curvature has a segmented appearance (fig.

97), which is due to the folds in the body wall. The pharynx and stomach are now united by a distinct tube, the oesophagus (figs. 97 and 98) and the digestive system is now completely formed and differentiated into the following regions: buccal pouch, pharynx, oesophagus, and stomach. The trophi or jaws are formed from the ventroposterior wall of the pharynx (fig. 100). The gastric glands are formed at the junction of the oesophagus and the stomach (figs. 103 and 104). A few of the muscles which are directly connected with the different organs are represented in figures 98, 101, and 102. Figures 96 to 101 show the gradual straightening of the embryo, and the migration of the mouth to its definitive position. A small part of the foot still persists.

The trochal disc with its cilia (fig. 99) is completely formed. Its relation to the buccal pouch is represented in figure 100, a horizontal section of the same stage. The invagination of the ectoderm to form the urogenital sinus (figs. 98 and 101) corresponds to the proctodaeum when an intestine is present. It is formed on the dorsal side of the foot near its base, but when the foot is completely absorbed the opening is considered as being on the ventroposterior end. Figures 102 and 103 represent horizontal sections of figure 101, taken near the ventral and dorsal sides, respectively. The body cavity at this stage becomes very prominent and the different organs within reach their definitive condition. Figure 104 represents an embryo which has reached its distinct adult condition, represented as a transparent object from the dorsal side. All of the more important organs are shown. The digestive tract has reached its complete development as it occurs in the parthenogenetic female. The cells of the ectoderm lose their boundaries and become a definite syncytial layer, and the ectoderm with its delicate smooth cuticula constitutes the body wall.

The early stages in the development of the digestive tract of the male and female are similar, but when the male embryo has reached the condition represented in figures 79 and 87, there is a temporary fusion of the cells of the wall of the pharynx with those of the entoderm as in the female. The cells which, in the female, produce the oesophagus, cease to divide in the male

embryo and have more the appearance of yolk cells. These cells gradually recede from the pharyngeal wall (fig. 5), and finally take up a position on the dorsal side of the embryo (figs. 2, 6). Figure 6 represents the adult condition of the digestive tract in the male at birth. The stomach in the female rotifer Asplanchna ebbesbornii ends blindly. There is no indication of an intestine in the early stages of embryonic life.

REPRODUCTIVE SYSTEM

The origin of the reproductive organs is directly associated with the entoderm and they arise from the derivatives of the mesentoblast. Thus, the digestive and the reproductive organs have a common origin. The differentiation of the reproductive system is very rapid, especially in the male, since they are sexually mature before birth. Practically all of the derivatives of the large cells $E^{2.1.1}$ and $E^{2.1.2}$ of the five large mesentodermal cells (fig. 67) contribute to the formation of the reproductive system. About the first indication in the differentiation of the digestive and reproductive regions is the appearance of darker granules in the reproductive portion. This differentiation is well marked during the early formation of the pharynx (figs. 76, 77, 79, and 87). These cells are indicated by heavy stippling.

The reproductive cells continue to divide and later become specialized into two groups or regions (fig. 90), one forming the vitellarium and the other the oviduct and uterus, which becomes continuous with the urogenital sinus (figs. 95 to 97). The differentiating vitellarium is at first a spherical mass of cells, but later sends out an arm right and left and finally becomes U-shaped (figs. 95 to 104). The vitellarium produces the yolk and supplies the egg with its granular and yolk content. Yolk production is well marked in those individuals that produce thick-shelled resting eggs. A small portion of the vitellarium, in the region of the oviduct becomes specialized into a rudimentary ovary (figs. 100 to 104), which contains a number of very minute cells. These cells enlarge, one at a time (occasionally two develop simultaneously), and become the mature ova. In the older female, at the close of the reproductive period, the ovary is very

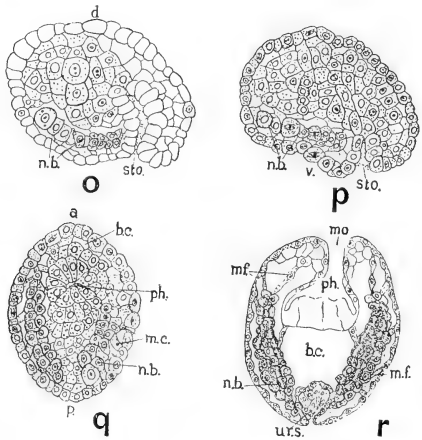
indistinct and finally is indiscernible. No vitellarium is formed in the male, but instead the cells are differentiated into the testis and the vas deferens, which are continuous with the urogenital sinus, which in the male is ciliated. These cilia are very active and aid in the passage of the sperm from the vas deferens into the uterus. In the female the upper end of the reproductive tube is very narrow and can be designated as the oviduct. The urinary bladder is formed by an evagination of the urogenital sinus.

THE EXCRETORY SYSTEM

The excretory system is ectodermal in origin. It arises from special cells, which become separated from the outer embryonic epithelial layer on its inner surface at the ventroposterior end (figs. 77, 79, 87, and 90). These cells act like teloblasts and, by a rapid proliferation of cells in an anterior direction, produce on either side of the ventral median line a mass of cells (text figs. o and p) from which the excretory tubules are formed. The cells of each band are arranged in definite rows. Later cavities appear within the cell rows; these become continuous from cell to cell and give rise to the lumen of the future forming tubules (text figs. q and r). The cells directly concerned in this process contribute to the tubule walls with a few persisting nuclei. Later, each band becomes differentiated into a system of tubules with nucleated walls (text fig. s). Text figure u represents the condition of the embryonic excretory tubules of the right side of the embryo, shortly before birth. Either half is composed of one main and two recurrent tubules. The three tubules are united at either end. The manner in which this union occurs varies in different individuals. Each tubule has a distinct, non-ciliated lumen with a nucleated wall. A number of small club-like evaginations are formed on the main tubule of either side. Their number and size vary. The maximum number found on any one tubule was fifty. The free ends of many of these evaginations are enlarged and contain cilia, which exhibit a constant flickering motion. These club-shaped organs constitute the so-called tags or flame cells. In their early formation the free ends

often show protruding cilia and communicate directly with the body cavity (text fig. s). In the later stages, however, the open tags are rare and exhibit closed ends with pending cilia (text fig. u). Tags are never formed on the recurrent tubules.

The systems of excretory tubules on either side do not communicate at their anterior ends, but at their posterior ends they connect with the urinary bladder. The urogenital sinus, as stated above, arises as a solid ingrowth of the ectoderm at the



Figs. o and p Median longitudinal sections, to show the early formation of the excretory system; note the nephridial bands on the ventral side within the ectoderm.

Fig. q Horizontal section of the same stage as above; the section shows the rows of nephridial cells and their connections with the extoderm at different intervals; these connections become the controlling muscle fibers.

Fig. r A horizontal section of a later state; shows the early lumen in the formation of the excretory tubules.

ABBREVIATIONS

a., anterior
b.c., body cavity
d, dorsal
m.c., muscle cell
m.f., muscle fiber
mo, mouth
n.b., nephridial band
ph., pharynx
sto, stomodaeum
ur.s., urogenital sinus
v, ventral

dorsal posterior end (text fig. r and fig. 98). The urinary bladder (pulsating vacuole) is formed by an evagination and enlargement of the inner end of this invagination (text figs. s and t). The walls of the urogenital sinus on either side, in its early differentiation, fuse with the posterior ends of the group of cells which form the excretory rudiments (text figs. r and s), and finally a direct communication is established between the pulsating

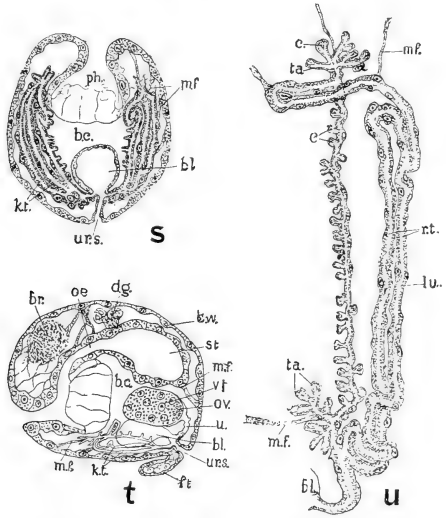


Fig. s Horizontal section of embryo, to show fomration of the excretory system, tags, urogenital sinus, and bladder.

Fig. t Median longitudinal section, to show the relation of parts.

Fig. u Completely formed excretory tubules from the right side of embryo at the time of birth.

ABBREVIATIONS

bl, bladder
br, brain
b.w., body wall
c, cilia
d.g., digestive glands
ft, foot
k.t., kidney tubules
lu, lumen
oe, oesophagus
ov, ovary
r.t., recurrent tubules
st. stomach
ta, tags (flame cells)
u, uterus
vt, vitellarium

vacuole and the tubules. At first the urinary bladder shows a distinctly nucleated wall (text fig. s), but later, as enlargement continues, the cells are no longer distinguishable and the wall becomes more membranous, as in the adult. Text figure t, an optical section of a fourteen-hour embryo from the left side, shows the relation of the different parts of the excretory system.

The movement of a finely granular substance within the lumen of the tubules is visible under high magnification. This flow of substance is due to the action of the cilia within the flame cells and the activities of the delicate fibers in the tubule walls. The constant rhythmical contraction and expansion of the urinary bladder contribute to the process of elimination of the excretory products. In the formation of the excretory system on the ventral side, its early rudiments do not lie free in the body cavity but are directly connected with the ectoderm at different points by means of cells, which originated from the ectoderm (text figs. q and r). These cell connectives later develop into the muscles, which connect the forming tubules with the body wall. These tubules at first are on the ventral side of the body cavity, but later, as their associated muscle fibers develop, the excretory tubules may occupy almost any part of the body cavity, depending upon the contraction and expansion of their controlling muscles.

NERVOUS SYSTEM

The brain is formed by a solid ingrowth or proliferation of cells from the ectoderm at the anterior end of the embryo, dorsal to the mouth (figs. 96 and 98). A small portion of the brain on the dorsal side gives rise to the rudimentary eye, which appears as a red pigmented body. The different intensities in the pigment formation of the eye can be recognized in the developing embryo. The origin of the nerve fibers can be distinguished in section (fig. 103). This figure shows the origin of the lateral and anterior nerves, which innervate the trochal disc. Each of the lateral trunks gives off a branch which runs nearly the entire length of the animal. These nerves are hard to demonstrate except in the close proximity to their origin.

Sense organs: The antennae are tubular outgrowths of the ectoderm with number of sense hairs projecting from the apex of each. In their early development, these hairs show a slight vibratory action, but when fully developed they are stiffer and firmer and serve as organs of touch. Delicate nerves extend from the brain to the different antennae. There are four antennae present, two at the anterior end and one on either side of the body in a dorsolateral position, nearer the posterior end (figs. 1 to 3, and 6).

POSITION OF ROTIFERS IN THE ANIMAL KINGDOM

The position of rotifers has been a subject of considerable controversy. Huxley ('51) suggested that they represent a primitive form and are preserved, with modification, in the larvae of molluscs, annelids, and other forms. Lankester maintained a similar view and considered the trochophore of worms and molluscs, when compared with rotifers, as possessing close relationships. Hartog held the view that the structure of the rotifers brings them into close relationship with the lower flat worms and with the more primitive larvae of the Nemerteans, the Pilidium, and that there is a striking resemblance in the structure and function of the different parts, when carefully compared. Thus the rotifers, he says, may be considered as a group apart, but probably representing an early offshoot from a free-swimming plathelminths (Rhabdocoele) with minor change.

Zelinka endeavored to prove that the course of development in the rotifer Callidina russeola, as well as other rotifers, shows affinites with the trochophore larvae of Annelida and Mollusca, and that the adult rotifers are in a sense persistent trochophore larvae. Balfour states that the trochophore larva is found in rotifers where it is preserved in the adult state, and that there is every reason to believe that the types with trochophore larvae, viz., the Rotifera, the Mollusca, the Chaetopoda, and the Polyzoa, are descended from a common ancestral form, and that it is also fairly certain there was a remote ancestor common to these forms and to the Plathelminthes. Other investigators

interpret the trochophore as representing a simplified form, an ancestor which, were it living to-day, would be classified as a ctenophore, and that the three distinct larval types, namely, Müllers larva, the Pilidium, and the trochophore, all represent, with more or less change of form, a group of ancestral Ctenophora, from which sprang the Polyclada, and through them all the plathelminthes, the Nemertfans, and the annelids.

Considering the life history of the different groups in question from the standpoint of resemblances and differences, the above phyletic scheme becomes a tenable one. If the rotifers represent a primitive or ancestral type, and are preserved, with modifications, in the larvae of annelids and other forms, what position in the phyletic scheme do they occupy, more especially when considered from the standpoint of cleavage and early development? The larvae of the annelids not only show resemblances to that of the rotifers, but, in addition, they possess in concentrated form the rudiments of the future adult annelid body. Also the mesodermal structure in the trochophore must undoubtedly represent the mesoderm of the ancestral type. The point at issue is, however, not so much what the completely formed larvae of annelids or the rotifers as such possess, but is there any parallelism in their development in reaching this point, which is of any phyletic value?

If cell homology have any significance, according to some writers we must conclude that the cells whose products are homologous must be themselves homologous, even though they may have the same or different origin and position in cleavage. Light on the systematic position of rotifers may be gained by comparison of their development with that of other groups. The studies on cell lineage no doubt are invaluable in determining phyletic relationships, as has been proved by results along this line of investigation. Characters of forms, as manifested during cleavage, in many instances are as constant as are anatomical characters in later stages, and must therefore be as truly inherited. And since coenogenetic changes may be supposed to affect the later stages of development first, we may expect to find earlier stages retaining longer their primitive characters. It

is true that the early development cannot be regarded as infallible in determining relationships in every case, yet it may be called to aid in solving genetic relationships of questionable forms.

It is with the above points in view that the early stages in the development of the rotifers, as compared with other forms, are emphasized as being of considerable phyletic importance in the determining of relationships.

In Asplanchna ebbesbornii, the early development shows such striking and accurate resemblances to that of the annelids, especially that of the fresh-water Bdellodrillus, that it seems almost impossible to think that such minute similarities could have arisen independently in their evolutionary history.

The shape of the eggs, the position of the polar body or bodies, and the early cleavage stages are almost identical. In the beginning with the one-cell stage the position of the cleavage spindle and the direction of the cleavage are the same, dividing the egg into two very unequal cells (figs. 7, 8, 16, and 17). The first cleavage plane occurs at right angles to the median longitudinal axis of the future adult. In the formation of the four-cell stage, the large cell divides first and very unequally, while the smaller cell divides equally. These two cleavages, taken together represent the second cleavage as it occurs in the annelids. The large cell D is posterior, B anterior, C right, and A left, with reference to the median axis of the future individual. The large cell D and its derivatives always divide first. The position of the four macroneres, as in Bdellodrillus, determine the orientation of the future adult organs of the rotifer (figs. 20 to 24).

The sequence of cleavage stages corresponds to that of the polyclades, nemerteans, and the annelids. Three generations of micromeres are formed, which contribute to all of the definitive ectoderm and the ectomesoderm (larval mesoderm of the annelids). After the formation of d^3, 3D gives rise to all of the entoderm and the mesentoderm, including the reproductive organs. In the rotifers, however, the entoderm and mesoderm are separated at a later stage in the cleavage of 3D, also 3A, 3B and 3C remain on the surface in the region of the blastopore and do

not invaginate with the large cell 3D, and thus take no part in the formation of the future gut, but, by a rapid proliferation, their products grow posteriorly and form the major portion of the foot, which is absorbed before birth. In the polyclade Planocera, as in the rotifers, 3D has the same fate; likewise, 3B, 3C, and 3A, with their derivatives, take part in the invagination, but do not contribute to the gut, and are later absorbed as food.

As stated, the sequence of cleavage in the formation of the quartettes in the rotifers is comparable to those of the polyclades and annelids, but the position taken by the resultant generations of cells is different. This difference, however, is adaptive. The cells, instead of remaining in a more spherical mass, are drawn out in an anteroposterior direction. The cleavage cells take up this early position in accordance with their later formation and the needs of the future animal. The rotifers, in their early development, possess characters which are common to both the polyclades and the annelids. In addition, however, they possess characteristics which are peculiar to the polyclades and others which are distinctly annelidian.

When rotifers are compared with the adult Dinophilus, some very striking points of resemblance are recognizable. In both the bands of cilia, which are the free-swimming organs of locomotion, represent the prototroch or remnants of it. The adult Dinophilus remains to a certain extent at the stage of the annelid larva or a stationary larva which has become sexually mature. Here the trochophoral characteristics persist in common with the worm like-form of the annelids.

In the rotifers the trochophore stage persists, and among some of the rotifers at least, it is the end or climax stage in development. Many of the rotifers, however, possess in addition a worm-like body and are capable of an annelid creeping motion, independent of the trochal cilia, which indicates to some degree a specialization in a definitely directed line. As previously stated, there is a distinct parallelism in the development of the two forms. The sequence of cleavage is the same, but there is a dissimilarity in the position taken by the cleavage cells. This difference is, however, adaptive. In the rotifers the

entire animal (trochal region, body, and foot), develop simultaneously, hence the position taken by the early cleavage cells to meet the future needs in the formation of the adult structures.

In Dinophilus the larval or trochophoral development is accentuated, and the future adult body at first exists in a concentrated form in a certain cell or group of cells at the future posterior end of the animal. The end result in either case is a distinctly formed animal, which exhibits many points that are common to both. The development of Dinophilus, however, is more like that of the annelids than of the rotifers, and could be considered as intermediate between the two groups of animals. Further, when considered from the standpoint of development, the rotifers exhibit many points of resemblance to the annelid larva, and no doubt represent a primitive type preserved as modifications in the annelid larva, but they cannot be regarded as an ancestral type from which the annelids sprung, but rather a form which represented the annelids at one period in their phylogeny.

Hence from cleavage and development of the various forms in question, the conclusion is reached that the polyclades, annelids, and rotifers must have originated from a common ancestral form comparable to that of the ctenophore.

The rotifers, although retaining their primitive condition, have departed somewhat from the common ancestral form and to some degree have become a specialized group. The annelids, on the other hand, have reached a much higher level in their evolutionary history and show affinities to the ancestral type during their larval development.

GENERAL SUMMARY

The undivided egg of Asplanchna ebbesbornii is nearly oval. Its median longitudinal axis, passing through the polar body or bodies, corresponds to the median longitudinal axis of the future adult. The first cleavage plane is at right angles to this axis and divides the egg into two very unequal cells. The second cleavage occurs at an angle of about 45° to that of the first. It divides the smaller cell equally and the larger very unequally. The larger cell divides first.

In the four-cell embryo, the large cell, D, is posterior, B anterior A left, and C right. The ectoderm is separated from the four macromeres by a series of three cleavages. The three quartettes of ectomeres share in the formation of the ectomesoderm. The macromere 3D gives rise to all of the entoderm, the reproductive bodies, and some of their directly associated muscle fibers. The macromeres 3A, 3B, and 3C, do not invaginate with 3D, but remain on the surface in the region of the blastopore and, by a rapid proliferation of cells, give rise to the major portion of the temporary foot, which is absorbed before birth.

Three kinds of eggs are formed: The female-producing and the male-producing, both of which develop parthenogenetically, and the thin- and thick-shelled resting eggs, which are fertilized and always produce females. There are two kinds of adult females, one produces females parthenogenetically and the other, males parthenogentically and resting eggs. Males and females or resting eggs and females are never produced by the same parent. The male and resting eggs have two polar bodies. When conditions are favorable, it requires about seventeen hours from the time of the formation of the polar body to the time of birth of the parthenogenetically produced males and females. The males and females are sexually mature at birth. The female increases four to eight times its size at time of birth. The males do not increase in size after birth, but perish almost immediately after copulation. Very few free-swimming males are found at any one time in the culture. The males are structurally degenerate. The development of the males and females is similar up to the late period in embryonic life. The cells of the stomach and the invaginated pharynx become continuous, but the oesophagus is never formed.

Gastrulation is epibolic. A central cavity is formed, which begins at the close of the formation of the third quartette of micromeres. The cavity is due to the shortening of the cells in the epithelial ectoderm, and is later occupied by the large cell, 3D. The gastrula is composed of a single outer epithelial layer, enclosing 3D, d^4, and d^5 with their later derivatives.

The digestive system is composed of the mouth, buccal pouch, pharynx, stomach, and the attached digestive glands. The stomach is strongly ciliated. There is no evidence of an intestine in embryonic life. The trochal disc is formed from early proliferated ectodermal folds at the ventro-anterior end. The embryo, at its greatest flexure, is completely bent on itself, and the embryonic foot extends anteriorly over the stomodaeum. The brain is formed by a solid ingrowth of ectoderm at the anterior end, above the buccal pouch.

The excretory system is formed on the ventrolateral sides of the median line from excretory bands or rows of cells. These bands begin at the ventroposterior end by the proliferation of special cells derived from the ectoderm. The adult system on either side consists of one main and two recurrent tubules. The main tube has a number of small evaginated tags bearing cilia on their interior.

I take this opportunity to express my gratitude to Mr. George T. Kline, the biological artist, for his helpful suggestions and execution of the drawings and lettering.

Columbia, Missouri, January 1, 1919

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PLATES

EXPLANATION OF PLATES

All drawings were made with the aid of the camera lucida under a magnification of 130 diameters. All whole-mount drawings were made from either the fixed or living egg and checked. Variations in the size of the surface views is due to a difference in the size of the eggs. The sections were not uniformly magnified. Stippling has been adopted for the sake of clearness.

REFERENCE LETTERS

a., anterior
a.a., anterior antennae
b.c., body cavity
bl., bladder
br., brain
bu.c., buccal cavity
b.w., body wall
c., cilia
c.c., cleavage cavity
cin., cingulum
c.l., cerebral lobes
coe., coelom
cut., cuticula
d., dorsal
d.a., dorsal arms
d.g., digestive glands
e., eye spot
e.cp., egg capsule
ect., ectoderm
emb., embryo
ent., entoderm
ft., foot
gn., ganglion
i.e.sh., inner egg-shell membrane
k.t., kidney tubule
l.a., lateral antennae
lu., lumen
m., muscle
m.c., muscle cell
mes., mesoderm
m.f., muscle fiber
mo., mouth
n.b., nephridial band
oe., oesophagus
o.e.sh., outer egg-shell membrane
ov., ovary
ovd., oviduct
p., posterior
p.b., polar body
ph., pharynx
pr., proctodaeum
p.s., polar spindle
r.t., recurrent tubules
st., stomach
sto., stomodaeum
ta., tag (flame cell)
tr., trochal disc (trochus)
tro., trophi
ts., testis
u., uterus
ur.s., urogenital sinus
v., ventral
v.m., vitelline membrane
vt., vitellarium
A, left macromere
B, anterior macromere
C, right macromere
D, posterior macromere
a^1, b^1, c^1, d^1, $d^{1.1}$, ect., first generation of ectomeres
a^2, b^2, c^2, d^2, $a^{2.1}$, etc., second generation of ectomeres
a^3, b^3, c^3, d^3, $a^{3.1}$, etc., third generation of extomeres
E, entoderm (mesentoblast)

PLATE 1

EXPLANATION OF FIGURES

1 Dorsal view of a female-producing individual.

2 Dorsal view of a male-producing individual, with nine male embryos at different stages of development.

3 Young female at time of birth, viewed from the left side.

4 Reproductive organs of a young female, dorsal view.

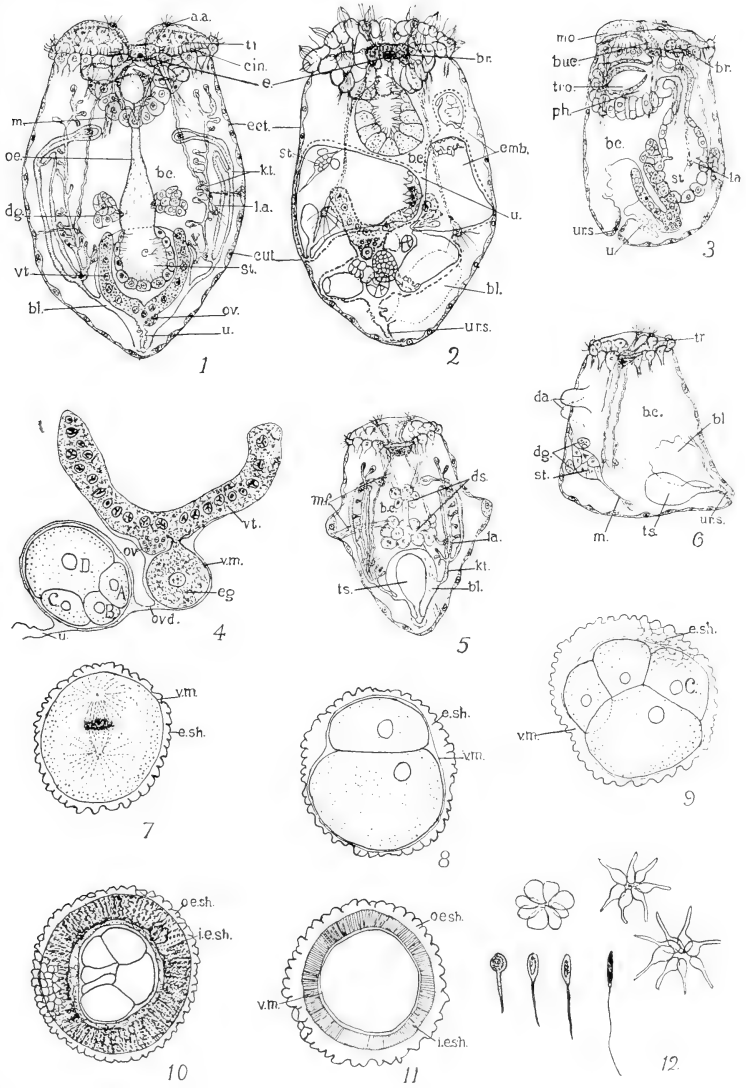
5 Male embryo, from dorsal view before birth; note the position of the digestive system (*d.s.*).

6 Male at time of birth, from right side; compare the final position of the degenerating digestive system with the preceding figure.

7 to 9 Thin-shelled resting egg, one- to four-cell stage; note the irregular structure of the egg membrane.

10 and 11 Thick-shelled resting egg; note the porous condition of the inner egg membrane.

12 Sperm cells in different stages of development.


a.a.
ti
cin.
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m.
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be.
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PLATE 2

EXPLANATION OF FIGURES

13 Parthenogenetic egg with polar spindle.

14 and 15 Same as the preceding after maturation.

16 Two-cell stage, dorsal view.

17 Two-cell stage, upper view, with asters for second cleavage.

18 Two-cell stage, ventral view, with CD dividing.

19 Four-cell stage, upper pole.

20 Four-cell stage, ventral pole.

21 Four-cell stage, dorsal view, showing the cells contracted.

22 and 23 Four-cell stage from left and dorsal sides.

24 Little later than figure 23, with d^1 cleavage-spindle forming; note the anterior extension of D in the process.

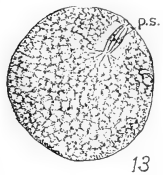
25 Five-cell stage, left side, to show lengthening of cells.

26 to 28 Five-cell stage, left and dorsal sides; note the abnormal flattening of cell d^1 in figure 28.

29 Five-cell stage, dorsal pole; shows the position and the rounded condition of the cells in preparation for the next cleavage.

30 Six-cell stage, dorsal view; note the position taken by d^1 and c^1 in the early third cleavage.

31 Seven-cell stage, dorsal view.



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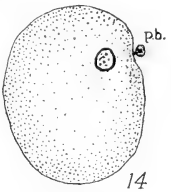

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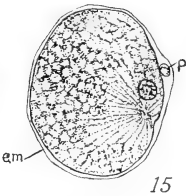

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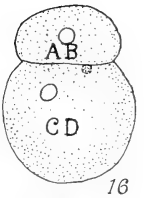

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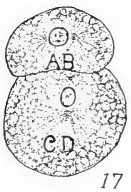

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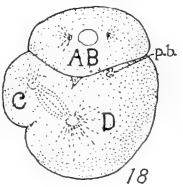

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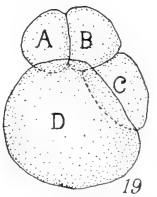

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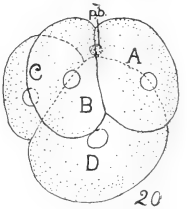

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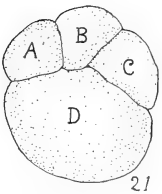

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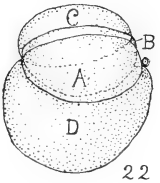

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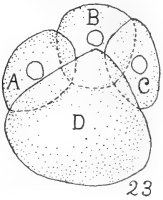

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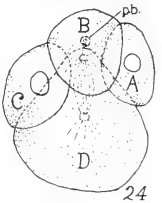

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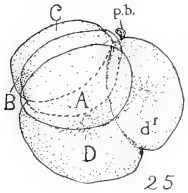

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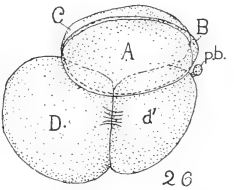

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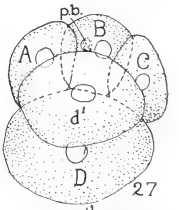

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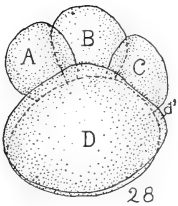

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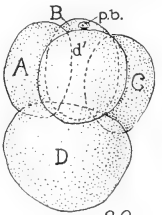

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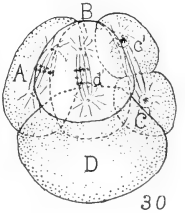

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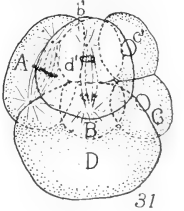

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PLATE 3

EXPLANATION OF FIGURES

32 Eight-cell stage, ventral view.

33 Eight-cell stage, dorsal view; the ectomeres are above instead of anterior to the parent cells as in figure 32.

34 Early nine-cell stage, upper pole (dorsal view).

35 Late nine-cell stage, ventral view.

36–37 Ten-cell stage, ventral and right sides.

38 Sixteen-cell stage, ventral view; the embryo is extended in an antero-posterior direction.

39 and 40 Sixteen-cell stage, the left side; D side is dorsal and dorso-posterior.

41 Sixteen-cell stage, right side.

42 Sixteen-cell stage, ventral view; all of the cells are represented; note the shape of the cells.

43 and 44 Sixteen-cell stage, dorsal view; the D derivatives are considerably elongaged transversely.

45 Nineteen-cell stage, right side; d^1, d^2, $d^{1.1}$ having divided; the eggs at this stage are transparent and the position of all the cells can be recognized.

46 Twenty-cell stage, right side; the extra cell is d^3 from D; the formation of d^3 is unusual.

47 Twenty-nine-cell stage, upper view; a continuation of figure 46 during the fifth cleavage; the A row of cells has not divided.

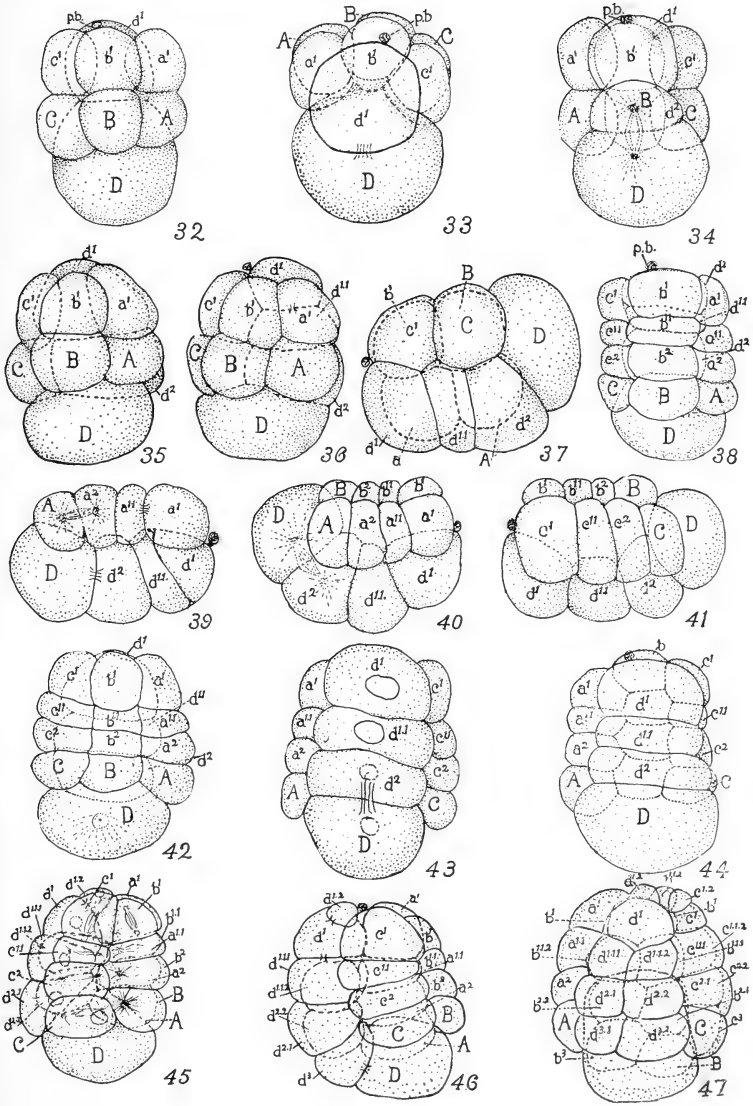
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PLATE 4

EXPLANATION OF FIGURES

48 Nineteen-cell stage, dorsal view, showing the exact position of all the cleavage cells.

49 Thirty-three-cell stage, dorsal view; the end of fifth cleavage; d^3 is present and has divided; the large cell D (E) has begun to invaginate.

50 Thirty-five-cell stage, dorsal view; the minute cells d^4 and d^5 are the additional cells formed.

51 Thirty-four-cell stage, ventral view.

52 Thirty-four-cell stage, posterior end of the embryo, to show the position of the closing blastopore.

53 Thirty-three-cell stage; same view as preceding figure.

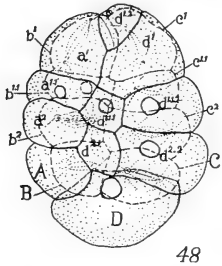
54 Later stage in the closure of the blastopore from the posterior end.

55 to 57 About fifty-cell stage, showing the invagination of the large cell E the central cavity into which E passes is indicated by fine stippling.

58 Sixty-four-cell stage, dorsal view, showing the first cleavage spindle of E.

59 Same as the preceding from ventral view.

60 Later stage in which E has divided.



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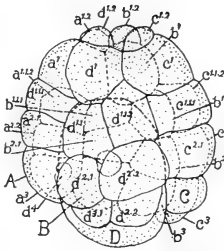

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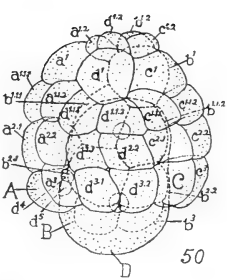

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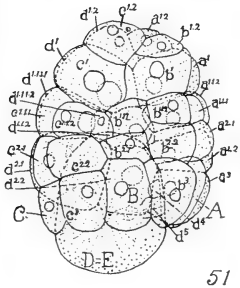

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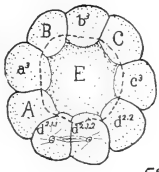

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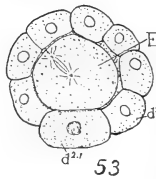

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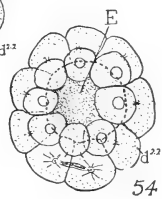

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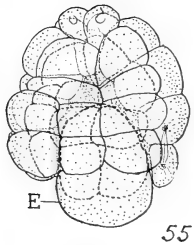

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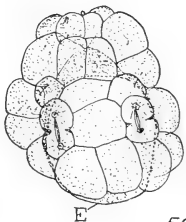

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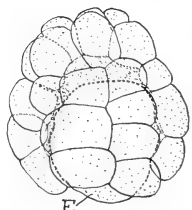

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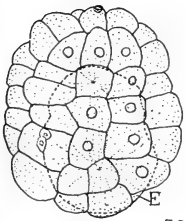

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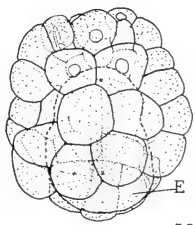

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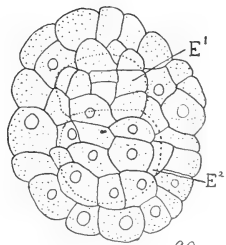

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PLATE 5

EXPLANATION OF FIGURES

61 Late cleavage, dorsal view, showing the preparation of E^2 for division.

62 A little later than preceding, showing three large entodermal cells; the embryo is considerably contracted and is more spherical.

63 Two-hundred-and-fifty-cell stage, dorsal view.

64 Same as preceding, in a contracted condition.

65 Embryo with the dorsal ectoderm not represented, to show E and its derivatives; the two posterior cells are in preparation for cleavage.

66 Stage little later than last; shows the five large interior cells; $e^{1.2}$ not represented.

67 Embryo viewed from the right side, the ectoderm not shown; the interior cells are slightly rounded and do not completely fill the space; the dorsal side is to the right of figure; the blastopore is closed.

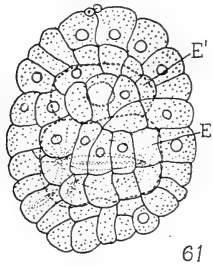
68 Dorsal view of embryo with overlying ectoderm not shown; ten large entodermal cells are present, five upper and five lower; the cavity represented is the future body cavity.

69 Same as the preceding, contracted, with the ectoderm represented.

70 An embryo showing the division of the five upper and five lower large entodermal cells; a few of the spindles are shown.

71 Late embryo from the right side showing the early formation of the stomodaeum and the temporary foot.

72 An optical section of embryo little later than last.



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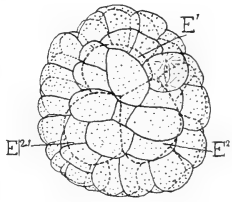

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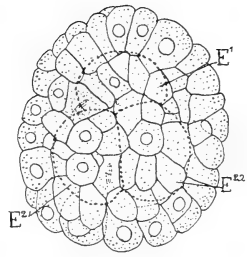

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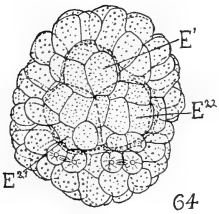

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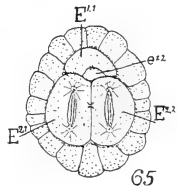

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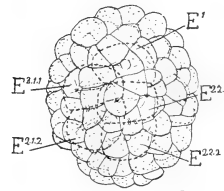

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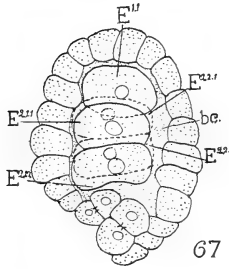

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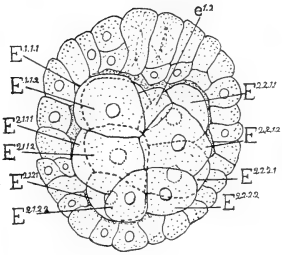

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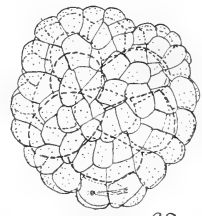
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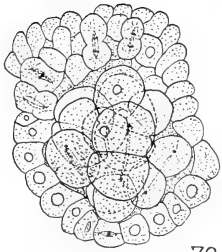
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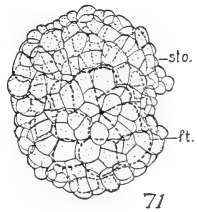

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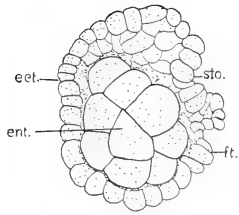

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PLATE 6

EXPLANATION OF FIGURES

73 Embryo, ventral view, showing position of stomodaeum and the beginning of the trochal folds (ectodermal proliferations).

74 Same as figure 73, right side; the unshaded portion represents the trochal prominence, especially near the ventral edge.

75 A median longitudinal section of the preceding figure.

76 A median longitudinal section of a twelve-hour embryo, to show the early formation of the foot and the differentiation of the inner cell mass in the formation of the digestive and reproductive systems.

77 A longitudinal section to side of median line, to show further differentiation.

78 Ventral view of whole embryo to show formation of foot and trochal folds.

79 Longitudinal section of preceding figure; the lumen of stomach is formed.

80 Horizontal section of figure 79, taken at *a–a*.

81 Embryo, ventral view, to show the approximation of the two ends; the lateral folds later become part of the trochal disc.

82 Horizontal section of figure 79 taken at *b–b*.

83 Embryo from right ventral side.

84 Same as the preceding, dorsal view, showing the condition of the ectodermal cells; this figure represents a three-hundred-cell embryo.

85 Embryo, ventral view, showing further differentiation of the foot and development of the lateral trochal folds.

86 Embryo, ventral view, showing the anterior and posterior folds of the trochal disc; note position of mouth.

87 Longitudinal section of embryo corresponding to figure 86.

88 Horizontal section of figure 79 taken at *c–c*.

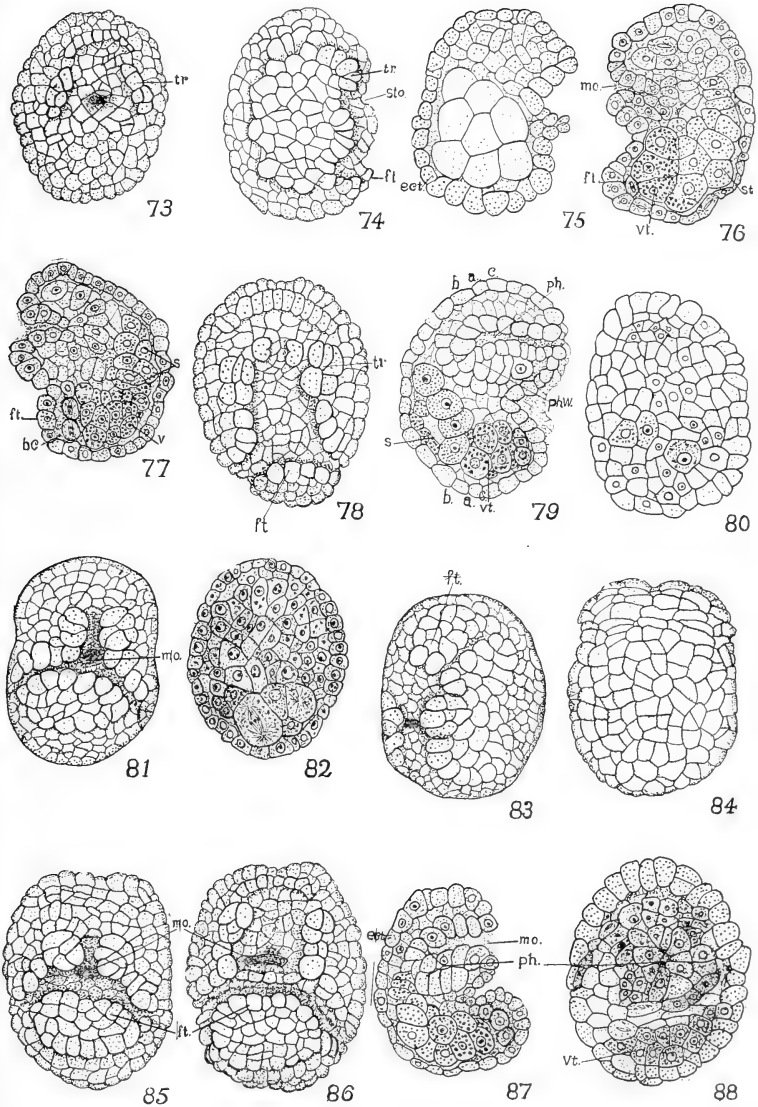
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PLATE 7

EXPLANATION OF FIGURES

89 Ventral view of embryo, showing the curvature in a ventral direction; the trochal disc is completely formed, but is very irregular; the ventral view at this stage corresponds to the ends of the embryo.

90 Longitudinal section of embryo represented in figure 89.

91 and 92 Embryos, ventral view showing the overlapping of ends; the foot at this stage is forked.

93 Embryo showing maximum curvature.

94 Embryo contracted into spherical condition.

95 Longitudinal section of figure 93 taken to the side of the median line.

96 A median longitudinal section of embryo passing through the brain region; note the position of the mouth; the embryo has begun to straighten.

97 Embryo from left side, showing maximum development of foot; the segmented appearance is due to the folded condition of the ectoderm.

98 Longitudinal section of figure 97; the digestive system is completely formed.

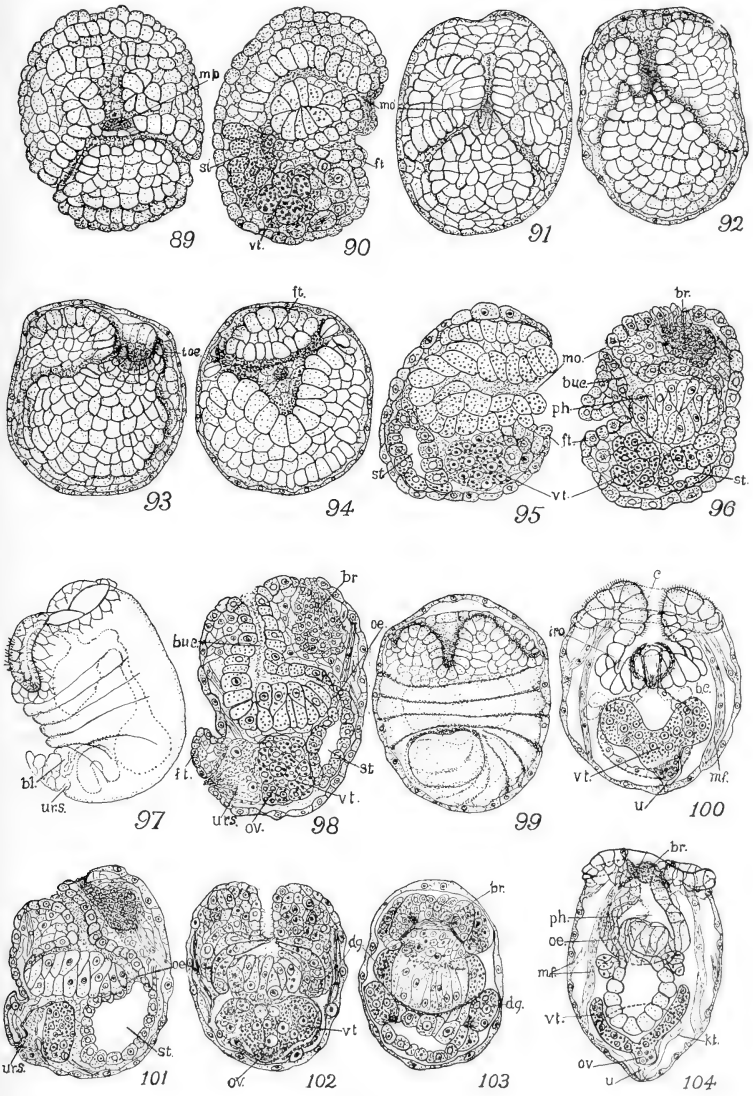
99 Embryo showing the further separation of the two ends and the shortening of the foot; the ciliated trochal disc is formed.

100 An optical horizontal section passing through the floor of the pharynx and the reproductive organs; note condition of body cavity and body wall.

101 Median longitudinal section of embryo later than preceding; the mouth has reached its definitive position at the ventro-anterior end.

102 and 103 Horizontal sections of preceding figure through dorsal and ventral sides, respectively.

104 An optical section of embryo at time of birth, showing the relative position and relation of different organs.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Resumen por el autor, Henry Carrol Tracy.
Escuela de Medicina Marquette.

El cráneo cupleoideo y su relación con el divertículo de la vejiga natatoria y el laberínto membranoso.

El divertículo precelómico de la vejiga natatoria de los cupleídos envía a la base del cráneo, a cada lado de la cabeza, una rama que ocupa un canal en los huesos exoccipital y proótico, y termina en dos vesículas dilatadas, una de las cuales está situada en una cápsula ósea del hueso pterótico, y la otra en una cápsula ósea del proótico. Los rasgos especializados del cráneo cupleoideo son los siguientes: El orificio auditorio situado entre los huesos basioccipital, pterótico y proótico; el orificio temporal colocado entre los huesos frontal, parietal, epiótico y pterótico, ocupado por una voluminosa expansión del canal de la línea lateral; el receso lateral (que communica interiormente con la cavidad craneal) está colocado entre los huesos esfenótico, pterótico y proótico y el ala lateral del frontal; la cápsula ósea esférica del hueso proótico se abre interiormente por medio de una ventana en forma de hendidura; una vesícula fusiforme en el hueso exoccipital; una cápsula ósea esférica en el hueso pterótico. Una parte del canal de la línea lateral está situada en el receso lateral, que sirve de canal libre para la transmisión de la presión ejercida por el agua desde el exterior a los espacios situados alrededor del laberínto. El ganglio trigémino-facial está situado en un receso del borde anterior del hueso proótico, en la superficie de la cápsula ósea de dicho hueso. Las ramas recurrentes de los nervios facial y vago forman un plexo intracraneal que parece inervar los canales de la línea lateral del receso lateral y el orificio temporal.

Translation by José F. Nonidez
Carnegie Institution of Washington

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THE CLUPEOID CRANIUM IN ITS RELATION TO THE SWIMBLADDER DIVERTICULUM AND THE MEMBRANOUS LABYRINTH

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THREE FIGURES AND FIVE PLATES (TWELVE FIGURES)

CONTENTS

INTRODUCTION

The remarkable relation existing between the ear and the swimbladder in certain groups of fishes has been known since the publication of the classic monograph of Weber ('20). The greater part of his account holds good even to the present day, and most of the current text-book statements and figures on

the subject have their source in his thorough and accurate description.

Weber shows that the ear-swimbladder relation is structurally very different in different cases; in the five groups of fishes in which he found this relation, he described three entirely different anatomical types. In later times, the list of species has been extended in which the swimbladder is known to be in relation to the membranous labyrinth; yet no one has been able to add any distinctly new types to those described by Weber. These three types may be briefly summarized as follows:

1. The primitive type. This type is essentially a relation of simple apposition of a precoelomic diverticulum of the swimbladder to the base of the skull. In many cases there is a membrane-covered foramen in the cranial bones between the swimbladder diverticulum and the membranous labyrinth. There are many varieties of this type. It is found in widely separated groups of fishes, e.g., Megalops, Notopterus, Sparidae, and species of Serranidae and Gadidae. Probably it occurs in other species not yet investigated.

2. The clupeoid type. In this type a diverticulum of the swimbladder in the form of a minute capillary tube extends into the head on each side, enters the skull, and ends in two large expanded vesicles which occupy an extensive and complicated cavity in the bones of the lateral and basilar region of the skull. The anterior vesicle comes into a definite relation with the utriculus. This arrangement, so far as is known, is found only in the Clupeoids and nearly related families.

3. The Weberian mechanism. In this type there is an articulated chain of small bones developed from certain anterior vertebrae, which serve to connect the anterior end of the swimbladder with the perilymph cavity. The two sacculi of the opposite sides are connected by a duct to which is attached a medium unpaired sac. This mechanism is found in the families Cyprinidae, Siluridae, Characinidae, and Gymnonoti. The existence of this specialized apparatus in these families suggests a common descent and has led to the formation of the order Ostariophysi.

The first of these types as it exists in different species has been described by various observers from the point of view of gross structure. The third type, that is, the Weberian apparatus, has been thoroughly investigated and, on its anatomical side, is comparatively well known.

Our knowledge of the second (i.e., the clupeiod) type of the ear-swimbladder relation has not greatly advanced since the work of Breschet ('38). Tysowski ('09) and de Beaufort ('09) are the only investigators to apply modern methods to the study of this mechanism. De Beaufort's work, however, is concerned with the whole group of Malacopterygii, and he reports little that is new regarding the essentials of this structure. Tysowski's paper is brief, and although it considerably advances our knowledge of certain features of the ear-swimbladder relation in Clupeoids, it contains certain morphological conceptions which are based on inadequate knowledge of the cranial structures in these fishes.

This investigation has been undertaken for two reasons, namely, that the American Clupeiods have been little studied and that many elementary features in these structures seem to be still matters of controversy. This paper will deal only with the bony structure of the skull in its relations to the swimbladder diverticulum and the membranous labyrinth. Certain details regarding the relations of the cranial nerves to these structures are also described.

Upon investigation it was found that the ear-swimbladder relation is essentially the same in all of the American Clupeoids examined. Therefore, in this paper, those parts of the cranium related to the swimbladder and the membranous labyrinth are described as found in a representative American species (Pomolobus pseudoharengus), with only incidental reference to the other members of this group. Another paper, soon to follow, will describe the membranous labyrinth and its relations with the swimbladder diverticulum.

The drawings for this paper were made by Mr. Leo Massopust, the department artist at Marquette School of Medicine.

HISTORICAL SUMMARY OF THE LITERATURE ON THE EAR-SWIMBLADDER RELATION IN CLUPEIDAE

According to Weber's description (1820), the anterior end of the swimbladder in Clupea harengus bifurcates and sends a small diverticulum into each side of the head. It enters the occipital region of the skull and passes anteriorly along a canal inside the bones at the base of the skull, and ends in two expanded vesicles each enclosed in a capsule of bone (globulus osseus). One of these, the anterior, lies in the basilar part of the 'temporal' bone, the other, in the lateral part of the 'temporal' bone.

The anterior bony capsule is nearly filled by the membranous bulla, but from the inner side it also receives a diverticulum from the vestibule of the membranous labyrinth which extends into it from the cavum cranii. The opening of the capsule, through which this diverticulum of the vestibule enters, is a large transverse fissure which opens into the cavum cranii. Inside the bony capsule the air-filled membranous vesicle of the swimbladder flattens against the surface of the distal end of the diverticulum and fuses with it. By the apposition of these two surfaces a septum is formed which stretches across the cavity of the capsule like a tympanic membrane and divides it into two parts. The edge of this septum is fixed to a 'cartilaginous' ring which is attached to the inner surface of the capsular wall. The posterior of the two bony capsules admits no diverticulum from any part of the membranous labyrinth, but is entirely filled by the membranous vesicle of the swimbladder.

Weber believed that he demonstrated an endolymphatic canal which runs under the brain and connects the vestibules of the two sides, (subcerebral canal). He also noted that in these fishes the wall of the membranous labyrinth is much thicker than the same structure in other fishes.

Breschet ('38) studied the ear-swimbladder relation in Clupea alosa. He describes accurately, and in more detail than Weber, the membranous labyrinth, the bony capsules, the cartilaginous tubes which enclose the swimbladder diverticulum, and the general relations of these structures to the rest of the skull.

He observed the lateral recess of the skull (which has been almost overlooked by later investigators) and its relation to the membranous labyrinth internally, and to the lateral line system (canaux excréteurs) externally. That this unusual relationship might have a functional significance, he evidently recognized, though the theory which he proposed is hardly consistent with modern physiological knowledge.

Breschet's conception of the perilabyrinthine spaces was remarkably accurate, considering the crude technical methods of investigation available in his time. He describes the ear as composed of tissue "ni absolument membraneux, ni absolument cartilagineux," fragile, a little elastic, and of the softness of rubber. He speaks of two 'commissures,' made up of the same tissue as the rest of the organ which hold the two ears together. One of these 'commissures' he describes as passing above the brain and connecting the utricular sinuses of the opposite sides; the other 'commissure' is the structure that Weber described as an endolymphatic connection between the two utriculi. Breschet, however, describes these commissures as not hollow like the membranous labyrinth, but composed of a 'tissu foliace,' or a membrane 'pliée ou roulée,' which is only a thickening of the tissues of the vestibule. He says: "La commissure inférieure n'offre point de véritable canal dans son interieur, pas plus que la commissure supérieure. . . . . La substance de la commissure s'indentifie avec les parois du vestibule, et ne doit être considérée que comme une expansion membraneuse de ces dernières." Modern technique proves Breschet to have been essentially correct in his conception of these structures. Nevertheless, certain investigators long after his time persisted in considering these structures as endolymphatic canals.

Breschet describes what he called an 'accessory bulb' attached laterally to the utriculus under the ampullae of the horizontal and anterior semicircular canals. He describes the utriculus as merely resting on the anterior capsule, thereby denying the existence of Weber's vestibular diverticulum.

Hasse ('73) investigated the ear and swimbladder diverticulum in Clupea alosa and Clupea harengus. He carries Breschet's

conception somewhat further in stating that the thickening of the tissue of the vestibule, that is, the 'knorpelartige Fassergewebe,' is only a dense connective tissue and is to be considered as a localized thickening of the dura mater. He denies the existence of the utricular diverticulum in the anterior bony capsule. He describes with more accuracy than previous writers the form and relations of the perilabyrinthine spaces. He is in error, however, in assuming that the endolymphatic duct of the two sides passes through the supracerebral canal to connect the two sacculi. In agreement with Breschet, he affirms the existence of the accessory bulb, but denies that it is supplied with a nerve twig or connected with the ear. He also mentions the relation between the tissue of the perilabyrinthine spaces and the lateral-line canals.

Retzius ('81) briefly describes the relation of the ear to the swimbladder in Clupea harengus. His work essentially agrees with that of Hasse, except that he was inclined to the opinion that the endolymphatic ducts end blindly as in other fishes. He also considers the supracerebral canal as merely a thickening of the dura mater. He denies the existence of the utricular diverticulum in the anterior bony capsule.

Mathews ('86), in an investigation of the skeleton of the British Clupeoids, has accurately described the bony canals and capsules in the herring (Clupea harengus), the pilchard (Clupea pilchardus), and the shad (Clupea alosa). He finds the posterior capsule in the pilchard differing from that of other species in that it is divided by a constriction. By cutting serial sections through the whole length of the swimbladder diverticulum of the herring, he demonstrates the tube to be open throughout.

Ridewood ('91) describes the ear-swimbladder relation in the British species, viz.: herring, pilchard, shad, sprat (Clupea sprattus), thwaite (Culpea finata), and anchovy (Engraulis encrasicholus). It is unnecessary to mention here the minor variations in structure which he found in these different species. Ridewood by this paper set back the course of investigation of the ear-swimbladder relation in the Clupeoids by reviving certain older conceptions which investigators were gradually show-

ing to be untenable. For example, he describes a utricular diverticulum in the anterior bony capsule, the existence of which no investigator since Weber has accepted; he describes a caecum of the utriculus ('bulbe accessoire' of Breschet), although Hasse had stated that this structure is not connected with the membranous labyrinth; he revives Weber's conception of the subcerebral canal, although no investigator since Weber has admitted it to be an endolymphatic connection.

In papers on the osteology of the skull in various lower groups of Teleosts, Ridewood ('04 a, b,) describes the form and relations of the bony capsules of the swimbladder in several genera of Clupeidae and related families. Essentially the same relations are found not only in all species of Clupeoids, but also in Chatoesus, Chirocentius, Dussumieria, Engraulis, Coila, Pellona, Pellonula, Pristingaster, and Hyperlophus. The posterior capsule is wanting in Clupea sprattus, and both anterior and posterior capsules are absent in Chanos salmoneus. In Coilia nasus there is also a large exoccipital capsule corresponding, in Clupea, to the fusiform enlargement of the exoccipital part of the bony tube. Megalops has a structure in the opisthotic bone which perhaps lodges a swimbladder vesicle. Ridewood also states that vesicles occur in the skull of Hyodon, Notopterus, and in the Morymoids.

The structure of the skull and its relations to the swimbladder in these last three genera are more fully described by Ridewood (04 c) and by Bridge ('00). The ear-swimbladder relation in these forms is unlike that of Clupeoids; it belongs with the first or primitive type as described above.

All the writers above discussed are to be classed together so far as their method, technique, and results are concerned. They relied entirely on dissection and injection methods and made little or no attempt to investigate minute relations. The next two writers, however, applied more modern and accurate methods.

Tysowski ('09) shows that both the subcerebral and supracerebral connections between the membranous labyrinths of the two sides are not endolymph tubes, but channels in the tissue

around the labyrinth. He demonstrates the fact that the utriculus does not send a diverticulum into the anterior bony capsule, and that the caecum of the utriculus (Ridewood) or the 'bulbe accessoire' (Breschet) is merely a three-sided thickening which projects from the wall of the utriculus.

Tysowski discusses at length the morphology of the dense tissue and the channels in it which surround and connect the membranous labyrinth of the two sides. He states that the most characteristic feature of the labyrinth in Clupeoids is the much thickened connective tissue of its walls. Structurally, this tissue is "eine fast homogene Grundermasse mit dunkleren fibrosen Streifen und den für das Labyrinthgewebe so characterischen Spindelzellen." This dense tissue is well developed on the upper and inner walls of the utriculus from which it goes over to the anterior wall of the sacculus. It is not limited to the wall of the labyrinth, however, but continues off from it quite independently; it extends under the brain, it bridges the space between the two anterior capsules, and becomes continuous with the corresponding tissue of the other side; it also crosses over the cerebellum from one superior sinus of the labyrinth to the other. Hasse had made out enough of these relations to enable him to suggest that this tissue is a condensation of the dura mater. But since Hasse's time Sterzi ('01) has shown that the dura mater is first differentiated in the Amphibia, and that in fishes the covering of the brain consists merely of an undifferentiated meninx primitiva surrounded by a perimeningeal tissue. Tysowski thinks that the true explanation of this dense tissue is that it represents a transitional stage in differentiation from the perimeningeal tissue into the envelope of the membranous labyrinth. The spaces in this tissue he considers as belonging to the membranous labyrinth, and hence properly called perilymphatic spaces. In these spaces are strands of elastic tissue which stain by the Weigert method; they doubtless develop by transformation of connective tissue. If we imagine these spaces developing around the whole labyrinth, we shall have spaces actually homologous with the perilymphatic spaces of higher forms.

An interesting structure which was entirely overlooked by all previous investigators except Weber, is a septum which divides the cavity of the anterior bony capsule into two unequal chambers, the inferior of which contains the membranous vesicle of the swimbladder. Tysowski describes this septum as a "fibrose, stark elastische Membran, deren deutlich längs verlaufende Fasern aus sonderbaren Kanälchen in der Knochenwand der Kapsel hervorzugehen scheinen." The periosteum from the inside of each chamber is reflected on to the corresponding surface of the septum.

To de Beaufort ('09) we are indebted for a comprehensive investigation of the swimbladder in the Malacopterygii. Of these fishes, the following genera present the clupeoid type of relation between the labyrinth and swimbladder: Clupea, Pellona, Opisthonema, Sardinella, Chatoessus, Engraulis, Dussumiera, Spratelloides, Coilia, and Chirocentrus. In this group, Pellona is the only genus in which the ear-swimbladder mechanism differs from the clupeoid type in any important details.

By applying modern technique, he was able to demonstrate definitely the non-existence of the utricular diverticulum extending into the anterior bony bulla (as described by Weber and Ridewood). He states that in sections the forward part of the utriculus with the macula acustica rests upon the opening of the bulla "davon durch den perilymphatische Raum getrennt wird." This relation showed in all his sections, both of older larvae and of young fishes. He also denies positively any direct connection between the two utriculi. The subcerebral canal is only a perilymphatic space. With regard to the supracerebral canal, he says: "Ich darf jedoch mit Bestimmtheit erklären, dass auch diese Verbindung nicht besteht, ebensowenig wie von Hasse beschriebene Verbindung des beiden Ducti endolymphatica."

Although de Beaufort adds little to our previous conceptions, he was the first to have a view of the structure of the Clupeoids and allied forms comprehensive enough to discuss the comparative anatomy of the clupeoid type of the ear-swimbladder relation. His discussion need not be repeated here, but we

may mention his suggestion that forms like Megalops may be transitional stages between the primitive type and the clupeoid type. As de Beaufort remarks, however, it is evident that the connection between the ear and swimbladder has developed independently in different groups of fishes. Apparent resemblances in these structures, as found in existing genera, indicate little regarding relationship or even the phylogenetic development of the ear-swimbladder relation in any given group of fishes.

De Beaufort describes stages in the embryonic development of these structures in the herring. He shows that the bony capsules are formed not by a 'hollowing out' process of the bones in which they are enclosed in the adult, but by a new process of bone development from connective tissue around the membranous vesicle of the precoelomic diverticulum.

TERMINOLOGY

The following terms are used throughout this paper in referring to the anatomical relations of the cranial structures, exoccipital (for occipitale laterale), epiotic, prootic (for petrosal): pterotic (for squamosal), opisthotic (for intercalar), and sphenotic (for postfrontal or postorbital ossification). Median designates the midvertical plane or structures lying within it; medial indicates the opposite of lateral, i.e., toward the middle. In referring to the channels in the tissue around the membranous labyrinth and the brain, the term 'perilabyrinthine' is used instead of perimeningeal or perilymphatic.

MATERIAL

This investigation is based on the study of the more common American clupeoid fishes. Adult specimens of the following species were examined: Alosa sapidissima (shad), Pomolobus pseudoharengus (alewife), Pomolobus aestivalis (summer herring), Pomolobus mediocris (hickory shad, fall herring), Brevoortia tyrannus (menhaden). The shad were bought in local markets; the specimens of the other species were obtained from the Marine Biological Laboratory, where they had been pre-

served in 10 per cent formalin. Sections of embryonic stages of Stolephorus mitchilli were also available.

The cranial structure in all these species is essentially the same, but the skull of P. pseudoharengus is less specialized than in the other species. Hence it may be used as a type form. All descriptions in this paper refer to this species, unless otherwise stated.

GENERAL RELATIONS OF THE SWIMBLADDER AND THE PRECOELOMIC DIVERTICULUM

The swimbladder, in the species investigated, has the form and relations typical of the Clupeoids. It consists of a fusiform, tubular organ of small caliber, which runs the length of the dorsal part of the visceral cavity. Posteriorly it tapers rather suddenly, and either ends blindly or opens to the exterior by communicating with the anus. Anteriorly it usually tapers slightly and terminates in a rounded end in the region opposite the third or fourth vertebra; cephalad to this it sends up into the base of the skull a bifurcated precoelomic diverticulum which brings the swimbladder into direct relation with the membranous labyrinth. The pneumatic duct is open in these fishes; it arises near the middle of the swimbladder and opens into the blind end of the V-shaped stomach sac.

The only important variations from the typical form in the species under consideration are in Stolephorus. In this genus a constriction near the middle of the swimbladder divides it into an anterior part which retains the typical tubular form and a posterior part which expands into a thin-walled chamber. The pneumatic duct springs from near the anterior end of this chamber. Similar relations are found in Pellona (de Beaufort, '09).

Through the greater part of its extent the swimbladder is covered on its ventral surface by peritoneum; dorsally, it is in contact with the body wall and separated from the vertebral column by the aorta and kidney; areolar tissue, in some places containing a large amount of fat, connects it with the strong aponeurosis which bridges across the intercostal spaces.

The relations of the anterior part of the organ are somewhat different. As it passes over the pericardial cavity, it comes to lie between the kidneys of the two sides. Here the intercostal aponeurosis sends off two strong layers, one of which runs ventral to the swimbladder and the other dorsal to it; each connects with the corresponding layer of the other side. This

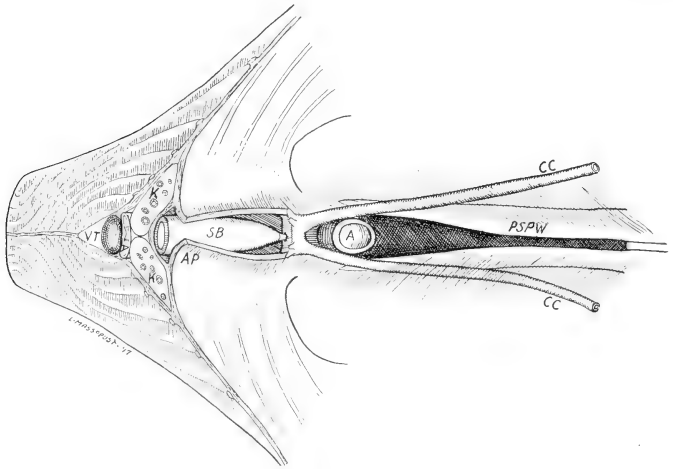

Fig. a Drawing to show relations of the anterior end of the swimbladder and the cartilage canals containing the precoelomic diverticulum and its branches (Alosa sapidissima). The structures are shown from the ventral side and in cross-section. Semidiagrammatic. Abbreviations on page 473.

part of the organ, then, is enclosed in an aponeurotic sheath of nearly tubular form, which runs anteriorly and attaches itself to the cartilage tube which encloses the precoelomic diverticulum (fig. a). The intercostal aponeurosis itself continues cephalad, to clothe the ventral surface of the musculature covering the occipital and basilar parts of the skull; other extensions of it pass dorsally to be attached to the vertebral column.

The precoelomic diverticulum is a tubular prolongation of the tunica interna of the swimbladder. There is some variation in

the details of this structure in different species. In Alosa (fig. a) and Stolephorus it passes forward a few millimeters from the anterior end of the swimbladder and enters a cartilage canal in which it almost immediately bifurcates. In Pomolobus, the branches come off directly from a slight bulbous enlargement of the tapering end of the swimbladder. Each of the branches,

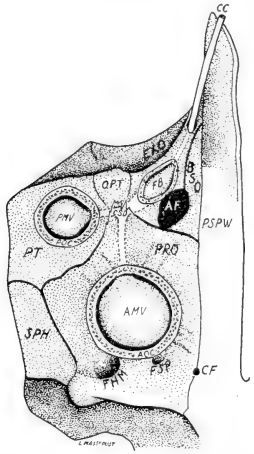

Fig. b. Drawing to show relations of the membranous vesicles of the precoelomic diverticulum to the bones at the base of the skull. Based on figure 8. The ventral segments of the bony capsules are represented as having been cut out. Diagrammatic. Abbreviations on page 473.

enclosed in a continuation of the cartilage canal, runs forward, one on each side, under the occipital portion of the skull and enters a canal in the exoccipital bone (fig. 2). The canal bulges somewhat here (P. pseudoharengus) to form a fusiform enlargement; it then passes forward to the angle where the exoccipital prootic and pterotic bones meet (fig. b). At this point, it gives off a lateral branch which enters a nearly spherical cavity in the pterotic bone which it expands to occupy, thus forming the pterotic or posterior membranous vesicle. The part of the pterotic bone which encloses this vesicle is the posterior or ptero-

tic osseous capsule, or bulla. The main branch of the diverticulum, from the angle between the bones above mentioned, continues forward in a canal in the prootic. In this bone the diverticulum ends by forming a membranous vesicle (anterior or prootic). The bone around the vesicle forms the anterior, or prootic bulla, or osseous capsule.

The cartilage canals in which the extracranial portions of the diverticulum are enclosed form a Y-shaped structure (fig. a). The stem of the Y is a median, unpaired structure; in Alosa it is a short, thick cylinder with its posterior surface forming a vertical transverse plane and facing the anterior end of the swimbladder; this surface is smooth and circular, and around its edge is beset with several large blunted spines. In Pomolobus, the posterior surface is concave. To these spines and around the margin of its smooth face is attached the aponeurotic sheath which invests the anterior end of the swimbladder. Anteriorly, this cylinder-like structure is continued off into two cartilage tubes which diverge from each other to form the arms of the Y which embrace the aorta between them. These tubes gradually diverge from each other, pass obliquely over the surface of the parasphenoid wings just in front of their tips, then over the aponeurosis which clothes the occipital musculature and extend cephalad to the side of the exoccipital bone. They are slightly flattened as they are applied to the surface of the aponeurosis; one of these in a shad measured 0.38 mm. in one dimension and 0.5 mm. in the other.

On the surface of the exoccipital bone is a groove (fig. 5) which begins toward the lower posterior angle of the bone and slants upward and forward. The groove gradually deepens as it passes upward, and finally ends in the opening of the canal through which the diverticulum passes. The ends of the cartilage tubes are very oblique and are applied to the sides of the groove and the edge of the mouth of the canal into which it leads. Thus the groove in the bone is made into a complete canal by the application to it of the oblique end of the cartilage tube, and through this canal the diverticulum of the swimbladder enters the exoccipital bone.

THE SKULL IN ITS RELATION WITH THE PRECOELOMIC DIVERTICULUM AND THE MEMBRANOUS LABYRINTH

In this connection, the sides and base of the cranium are of particular importance; description of other parts of the clupeoid skull may be found in the papers of Mathews ('86) and Ridewood ('04).

The clupeoid cranium, although highly specialized as to certain features, conforms in its more essential osteological characteristics to the general structure of the skull of the lower teleostean groups (pl. 1). The axis of the posterior part of the skull is formed by the basioccipital, which articulates posteriorly with the vertebral column, anteriorly with the medial plates of the prootic of the two sides, and laterally with the exoccipital bones of the two sides. Lateral to the exoccipital and the prootic bones, and forming the most lateral part of the occipital region of the cranium, is the pterotic bone. The anterior medial cranial floor, formed chiefly by the two prootics, is completed by the basisphenoid (figs. 7 and 8) with the hypophysial foramen intervening between that bone and the median articulation of the medial plates of the two prootics. The extreme lateral part of the cranium anteriorly is formed by the sphenotic which articulates with the lateral edge of the prootic bone. The posterior osseous capsule, containing the posterior membranous vesicle of the swimbladder diverticulum, is contained in the pterotic bone; the anterior osseous capsule containing the anterior membranous vesicle forms a considerable part of the mass of the prootic bone (fig. 5).

These bones may now be described in detail.

1. The basioccipital bone

The condylar end of this bone is circular in form where it articulates with the first vertebra. The body of the bone is hollowed on its lateral surface in such a manner that it consists merely of a thin median plate separating two lateral concavities; small lateral wings slightly deepen these concavities (figs. 4 and

12). A conspicuous notch in the edge of these wings anteriorly forms part of the margin of the foramen auditivum. The lateral concavities in the sides of the bone form the median and ventral walls of the occipital portion of the saccular cavities (fig. 3).

The ventral side of the body of the bone forms a narrow, longitudinal, slightly concave surface bounded on each side by a broad ventrally projecting plate of bone (figs. 8 and 11). This surface and the ventral plates form the roof and part of the sides, respectively, of the occipital portion of the eye-muscle canal.

The external surface of the bone is formed chiefly by the external surface of the ventral plates and to a slight extent by the ventral surface of the lateral wings, joined by a few roughened trabeculae of bone.

2. The exoccipital bone

For descriptive purposes, we may consider this bone as composed chiefly of two flattened masses; one forms a part of the vertical posterior face of the skull, at the sides of the foramen magnum (fig. 4), the other forms the part of the ventral surface of the skull next to the basioccipital (fig. 5). These are continuous posteriorly with a thin, curved plate of bone which is applied to the lateral and dorsal sides of the condylar part of the basioccipital. The vertical part of the bone has laterally on its outer surface a rounded projecting ridge which is continuous above with a similar ridge on the epiotic (fig. 1). This ridge is the prominent angle where the posterior surface of the skull meets the ventral and lateral surfaces, and it lodges the posterior semicircular canal of the membranous labyrinth.

Arising obliquely from the cerebral surface of the posterior portion of the exoccipital is a thin triangular plate (*TPEXO*, fig. 4), which passes medially to meet the corresponding plate from the other side just over the dorsal edge of the median, vertical plate of the basioccipital where a synchondrosis unites the three contiguous edges of bone (fig. 12). Anteriorly the plate tapers to a point which ends in the middle line just back of the prootic bone (fig. 3); posteriorly it extends to the condylar

part of the basioccipital. It thus forms the roof of the hinder part of the saccular cavity. The plates of the two sides exclude the basioccipital completely from the foramen magnum and the cerebral cavity, and form a trough in which the brain stem rests. Near the anterior edge of the triangular plates are two oblique foramina (fig. 4); one, anterior to the other and much the smaller, opens to the upper lateral part of the saccular cavity and gives passage to the ninth nerve; the other is the entrance to a canal which is directed obliquely backward and outward and forms the exit of the tenth nerve. Posteriorly are the exits of the two occipital nerves.

The ventral part of the exoccipital bone forms a considerable portion of the floor and sides of the saccular cavity. Its most conspicuous feature is a small fusiform bulla through which the diverticulum of the swimbladder passes. Dorsolateral to that structure is the large oval opening of the canal of the tenth nerve; medial to it and almost at the edge of the auditory foramen is a small opening for the ninth merve (fig. 5).

3. The prootic bone

The conspicuous feature of the prootic bone is the large spherical bulla or osseous capsule which lodges the anterior vesicle of the swimbladder diverticulum. Looking first at the cerebral surface of the bone (figs. 6 and 7), one observes that it is thick and massive medial to the bony capsule (medial plate) and forms with the corresponding part of its fellow a rounded ridge of bone which extends transversely across the floor of the anterior part of the cerebral cavity like a raised threshold. The sixth nerve passes vertically through this part of the bone to the eye-muscle canal below (figs. 6 and 8). Posteriorly, on the cerebral surface of the bone, just behind the capsule, is a deeply concave depression which, in the articulated skull, is continuous with the dorsolateral concavity of the basioccipital and completes the saccular cavity anteriorly.

Lateral to the osseous capsule, two bony plates are given off (fig. 6). The lower plate, the lateral wing of the prootic, is con-

fluent with the posterior part of the bone around to the saccular cavity; it extends horizontally and ends in a crescent-shaped margin, forming part of the floor of the lateral recess which will be described later. The upper plate of bone is divided into two laminae; it extends backward, upward, and outward from the lateral surface of the bulla itself. From its shape it may be designated as the falciform process. It is continuous in front with the lateral wing of the prootic, with which it makes a round open angle; its posterior edge is free; its dorsal edge misses reaching the pterotic bone by a very narrow interval (pl. 2) which, in the fresh skull, is bridged over by the lateral mass of cartilage retained from the primordial cranium (fig. 8). From the under surface of this process, forming with it a rounded angle along its line of origin, the lower lamina of bone is given off; this may be called the inferior lamina of the falciform process (*ILF*). Posteriorly, this lamina articulates with a plate of bone from the pterotic, but, below, is free and curved so as to resemble half of a low broad arch (fig. 4). Between the two laminae are included the inferior end of the anterior semicircular canal and its ampulla.

Anterior to the osseous capsule, the prootic extends forward to articulate with the alisphenoid. Near the center of this part of the bone is a deep notch (fig. 6), which extends down to the rounded surface of the bulla, and which, when articulated with the alisphenoid, forms the foramen for the exit of the trigeminofacial nerve complex.

The anterior or orbital surface of the prootic is formed by the smooth surface of the osseous capsule surrounded by projecting bony processes in such a way as to form a broad shallow fossa (ganglionic fossa). The capsule retains its spherical form; the bony processes around it have the appearance of being molded on to it. The fossa contains the ganglia of the trigeminofacial complex. Inferiorly, a sharply projecting ridge separates the orbital from the ventral surface of the skull. Medially, a pillar of bone bounds this surface and forms the lateral margin of the opening of the eye-muscle canal. A deep recess extends from the fossa behind the pillar; from this recess the ramus palatinus VII gains access to the eye-muscle canal (fig. 8, *RP VII*), through a

small, ventrally directed foramen; from it also the ramus sympatheticus and the ramus pretrematicus for the pseudobranch pass in a bony canal over the anterior medial surface of the bony capsule to the ventral surface of the skull (fig. c).

Lateral to the ganglionic fossa a rather thick process of bone articulates with the sphenotic. The orbital surface of this process has a large groove for the maxillomandibular nerve. The base of the process is penetrated by a canal which passes laterally

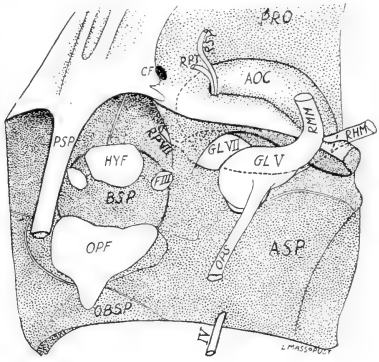

Fig. c Ventral surface of the middle region of the skull, showing the relations of the trigeminofacial ganglia and their branches to the prootic bone. Based on figure 7. Diagrammatic. Abbreviations on page 473.

over the anterolateral surface of the osseous capsule from the ganglionic fossa to the ventral surface of the skull. Through this canal the hyomandibular ramus of the facial nerve is conducted to the inside of the gill cover.

The relations of the ganglionic fossa recall the 'trigeminofacial chamber' of Allis ('03) and suggest the possibility of the homology of the capsule with a portion of that chamber. Knowledge of the development of the clupeoid skull is necessary, however, before this suggestion can profitably be considered.

On the ventral surface of the prootic bone, the medial plate is separated from the remainder of the bone by the broad, thin

ventral plate (*VPRO*), which articulates posteriorly with the ventral plate of the basisphenoid and with the posterior wings of the parasphenoid below, to form the lateral wall of the eye-muscle canal (fig. 8). This plate passes back from the medial pillar on the orbital surface of the bone across the ventral surface of the bony capsule in such a way that a small segment of the capsule is exposed to the eye-muscle canal (fig. 8). The foramen for the entrance of the carotid artery lies between the ventral plate of the prootic and the parasphenoid wings. The articulated medial plates of the prootics are joined with the basisphenoid in front and the basioccipital behind, and thereby form the roof of the eye-muscle canal. The ventral surface of the remainder of the prootic bone forms a large part of the ventral surface of the cranium. It is mainly taken up by a segment of the osseous capsule which protrudes from the middle of its surface. On each side of the front part of the capsule is a foramen through which the branches of the facial nerve mentioned above make their exit.

It is evident from the above description that a segment of the prootic capsule projects into all the cavities of which the prootic bone forms a part. About a third of the surface of the capsule contributes to the cerebral surface of the cranial floor; a segment faces the orbit where it forms the floor of the ganglionic fossa; about one-fifth of its surface protrudes from the ventral surface of the cranial floor; a small segment is presented to the lateral recess and another to the eye-muscle canal. A portion of the cerebral segment limits the saccular cavity anteriorly. The curvature of these segments is very little altered by its attachment to the parts of the bone in which it lies.

There are two openings in this capsule; the posterior (fig. 5) is the mouth of the canal through which the swimbladder diverticulum enters the interior of the capsule. The prootic portion of this canal begins at the posterior lateral angle of the prootic and proceeds obliquely through the thick part of the bone lateral to the saccular portion of the auditory recess (fig. 10).

The other opening or fenestra is in the cerebral segment of the capsule in that part of its curvature which looks posteriorly

(figs. 3, 4 and 6). In consists of a narrow transverse slit, slightly crescent-shaped in some species. Its edges, at its lateral end and sides, protrude upward slightly to form a raised lip around its margin. This lip gradually becomes lower toward the medial end of the fenestra where it is flush with the surface of the surrounding bone. The utriculus rests on the edges of this lip to which it is attached by delicate connective-tissue fibers.

The cavity of the capsule is subdivided into an upper and a lower chamber by a nearly horizontal septum of elastic tissue, the elements of which are in the form of flattened plates (figs. 5 and 9, *SC*). Each surface of this septum is covered by a reflection of the periosteum from the inner surface of the bone of the corresponding chamber. The septum is closely attached to the bone by means of radial connective-tissue fibers which pass from the elastic plates of the septum into minute canals in the bone substance. The lower chamber contains the anterior membranous vesicle of the swimbladder. The upper chamber was erroneously supposed by Weber and Ridewood to be occupied by a diverticulum from the utriculus; it is merely a tissue space which communicates with the subcerebral perilabyrinthine canal through the fenestra.

4. The pterotic bone

This bone forms the lateral part of the posterior portion of the cranial wall. It is a relatively large, massive bone in which is imbedded the posterior osseous capsule (figs. 3 and 11) containing the posterior vesicle of the swimbladder diverticulum. The lateral surface of the pterotic is partly covered anteriorly by a thin scale of bone which is an extension of the lateral wing of the frontal bone (fig. 1, *LWF*); posteriorly, the lateral surface is deeply indented by the lower portion of the epiotic fossa (*EPF*). The posterior osseous capsule protrudes into the epiotic fossa and also exposes its surface slightly on the face of the bone below the fossa. The lateral semicircular canal encircles the bony capsule near its equator.

The ventral surface of the pterotic is triangular and exhibits a small segment of the posterior osseous capsule (fig. 5, *POC*) lateral to which there is a shallow groove (deepened by cartilage in the fresh skull) which forms the posterior half of the hyomandibular articulation.

The greater part of the medial surface of the bone is deeply beveled so as to face nearly medially, and roughened for the attachment of the lateral cartilage plate (figs. 4 and 10). The bone articulates posteriorly with the upright posterior part of the exoccipital. A small vertical surface of the bone lies anterior to this articulation and makes an open rounded angle with the confluent surface of the exoccipital; through this angle the superior sinus of the membranous labyrinth passes vertically. A deep recess below extends back between the two parts of the exoccipital and lodges the inferior end of the posterior semicircular canal and its ampulla. This recess is separated medially from the saccular cavity by the canal for the vagus nerve. In front of the vertical medial surface is a cone-shaped recess which contains the ampulla of the lateral semicircular canal.

Anteriorly, the body of the bone juts out over its base to meet the backward projecting laminae of the falciform process of the prootic. To the angle between these laminae, the pterotic presents an obliquely excavated surface and thus forms a chamber for the reception of the lower end of the anterior semicircular canal and its ampulla. The floor and outer wall of this chamber is formed by the apposition of a thin curved plate of the pterotic bone to the inferior lamina of the falciform process. Superiorly, as has been mentioned above, the edge of the falciform process does not quite meet the pterotic: the irregular slit thus formed (figs. 3, 4 and 6) is closed by the lateral mass of cartilage which is penetrated by the anterior semicircular canal. Below the chamber the free edge of the projecting part of the pterotic is continuous with the edge of the inferior lamina of the falciform process and forms with it a low arch under which the utricular part of the auditory recess communicates laterally with the lateral recess.

5. The sphenotic bone

This pyramid-shaped bone forms the extreme lateral part of the anterior portion of the cranium (figs. 1, 2, and 9, *SPH*). The ventral part of the bone articulates posteriorly with the pterotic. Its upper part does not meet the pterotic posteriorly and the interval between them is bridged by the lateral wing of the frontal (fig. 3). Medially, the sphenotic bone articulates with the prootic and alisphenoid; laterally and dorsally, it is overlapped by the lateral wing of the frontal bone. The bone is deeply excavated posteriorly, so that a cavity occupies a large portion of its body. This cavity shares in the formation of the lateral recess.

6. The basisphenoid bone

This bone is a thin, slightly curved, median plate of bone, articulated between the front parts of the two prootics (fig. 7, *BSP*). It forms most of the floor of the hypophysial fossa and its posterior edge is the anterior margin of the hypophysial foramen. A deep notch in the posteriolateral margin of each side is converted into a foramen by articulation with the prootic and gives passage to the oculomotor nerve. The anterior edge of the bone forms the posterior margin of the optic foramen.

7. The opisthotic bone

This bone is almost rudimentary in the Clupeoids. It is a somewhat rectangular scale-like bone overlying the articulation between the exoccipital and pterotic bones (figs. 5 and 11, *OPT*). It has no relation to the membranous labyrinth or swimbladder diverticulum.

8. The lateral wing of the frontal bone

This is a thin scale of a bone extending down over the lateral face of the skull (fig. 1, *LWF*). Near its origin from the main part of the frontal, the posterior edge of the lateral wing forms the anterior margin of the temporal foramen; the anterior edge

of the wing joins the orbitosphenoid and alisphenoid. The distal end of this process flattens to form a thin lamella of bone which fits closely over a part of the adjacent surfaces of the pterotic and sphenotic bones on the lateral face of the skull. By bridging over the interval between these bones it helps to form the lateral wall of the lateral recess.

The lateral wing of the frontal bone serves chiefly as a bony sheath of the supra-orbital canal of the lateral line system. In the distal third of the wing, the inner wall of the bony sheath is deficient, so that here, at its origin, the supra-orbital canal is neither on the surface nor enclosed in a bony sheath, but lies in the lateral recess of the cranium (fig. 9, *LLC*). Two large foramina in the extreme distal end of the bone give passage to the infra-orbital and hyomandibular canals.

9. Remains of the cartilaginous cranium

Extensive unossified remains of the chondrocranium are retained in the adult, particularly in the orbital and nasal regions. There is also a thick triangular plate in the cranial roof and a large lateral plate which lines the cranial wall above the auditory recess. In the basal and occipital regions cartilage is limited to the synchondroses of the various articulations.

The lateral cartilage plate is an important structure in the auditory wall in Pomolobus pseudoharengus but is limited to the neighborhood of the anterior semicircular canal in most other species. Anteriorly, it lines the lateral wing of the frontal bone in its proximal part; further back it overlies the beveled medial surface of the pterotic (fig. 10) and nearly the whole medial surface of the epiotic (fig. 8, *LPC*). Owing to a deficiency in the lateral part of the epiotic bone, the plate contributes to that part of the exterior surface of the skull which lies at the bottom of the epiotic fossa (fig. 11). It completes the roof of the chamber containing the ampulla of the anterior semicircular canal by bringing across the interval between the falciform process and the opposed edge of the pterotic bone. The anterior semicircular canal passes through this interval and traverses a

canal in the cartilage plate until it reaches the edge of the epiotic where it passes under a hook-like process from that bone and emerges to the cranial cavity. The cartilage plate also contributes a small part to the roof of the lateral recess.

10. The lateral recess

This is an almost isolated cavity between the sphenotic, pterotic, and prootic bones and lateral wing of the frontal. It communicates medially with the utricular portion of the auditory recess through the low arch under the falciform process. The only other openings to this recess are through the foramina for the lateral-line canals in the lateral wing of the frontal bone, i.e., through the foramina for the exit of the suborbital and hyomandibular lateral-line canals and through the channel for the supraorbital canal as it arches up over the eye. By means of these canals it communicates immediately with the exterior. The recess contains a large sac-like expansion of the lateral-line canal from which the above-mentioned canals are given off. The rest of the recess is filled with a reticular-like connective tissue which is continuous through the arch with the perimeningeal tissue in the auditory recess. In this tissue is a large perilabyrinthine space limited by a well-defined marginal membrane (fig. 9, *PS*). The tissue is traversed by a recurrent lateral-line branch of the facial nerve which gains access to the recess through articulation between the prootic and sphenotic bones (fig. 3).

11. The temporal foramen

This is a characteristic feature of the clupeoid skull. It is a very large foramen in the upper part of the temporal fossa of the skull between the frontal, parietal, epiotic, and pterotic bones (fig. 1, *TF*). It is filled with adipose tissue which is continuous with, and apparently a part of, the perimeningeal tissue within the cranium. Previous writers have overlooked the fact that imbedded in this tissue, and nearly coterminous with the margin of the foramen, is a large bay-like expansion of a lateral-line canal.

THE CRANIAL NERVES

The relations of the cranial nerves in clupeoids correspond in general to those existing in other teleosts. The branches of the nerves from the third to the tenth correspond closely to those described by Herrick ('99) for Menidia. There are, however, interesting special relations of certain branches of these nerves.

The third nerve makes its exit from the cranium through the foramen between the basisphenoid and prootic bones; the fourth nerve penetrates the center of the alisphenoid bone (fig. 8); the sixth nerve gains access to the eye-muscle canal by penetrating the medial plate of the prootic bone.

The ganglia of the trigeminofacial complex lie in the ganglionic fossa and give off branches which pass through the prootic bone across the surface of the bulla (fig. 3). In addition to the branches mentioned above (p. 457), a recurrent branch is given off from the lateral part of the ganglion. It immediately divides into two rami which pass laterally to the back of the ganglionic fossa and through the articulation between the prootic and sphenotic bones, and enter the lateral recess of the skull. The more lateral of these rami courses obliquely across the floor of the recess to its posterior lateral corner where it supplies the hyomandibular lateral-line canal (fig. 3, *RVII*). The medial ramus curves gradually upward through the recess and penetrates the lateral plate of cartilage, thus entering the perimeningeal tissue (fig. 9) of the cranial cavity; it continues upward through this tissue, slanting slightly backward, and appears to form a plexus with the recurrent branch of the vagus. It also contributes to the supply of the sac-like expansion of the lateral line canal which occupies the temporal foramen. The medial branch has a course similar to that of the ramus recurrens facialis which Herrick describes in Menidia as made up of communis fibers.

In sections of the head of adult Pomolobus, I was able to trace branches to the bay-like expansions of the lateral-line canal as described above. These bays are so large that they may not function exclusively as lateral-line structures. The termination in them of the recurrent facial branches is therefore not neces-

sarily inconsistent with Herrick's account of these branches in Menidia.

The auditory nerve pierces the subcerebral plate of perilabyrinthine tissue (fig. 8, *VIII*) and divides into a saccular and a utricular division. The saccular division passes backward under the triangular plate of the exoccipital bone to the inner side of the sacculus. Under the edge of this plate it gives off a branch which passes over the sacculus to the posterior ampulla. The utricular division divides into branches which supply the ampullar organs of the anterior and horizontal semicircular canals and the three divisions of the macula acustica utriculi. The two branches to the ampullae are long; between them lie the two short branches to the posterior and middle divisions of the macula; the anterior division of the macula is supplied from the anterior ampullar branch.

The glossopharyngeal and vagus nerves leave the medulla together. They emerge from the skull through the exoccipital bone, though by different routes. The vagus passes through the vagus canal; the glossopharyngeal nerve passes through its foramen in the triangular plate of the exoccipital (fig. 4), traverses the upper part of the saccular portion of the auditory recess, passes under the posterior ampulla, and penetrates the skull just above the margin of the auditory foramen. The fusiform bulla lies between the exits of these nerves. The glossopharyngeal nerve gives a large communicating branch to the vagus as it passes through the auditory recess and receives one from it outside the skull. The vagal branch to the second branchial arch has a separate ganglion.

Just before entering its canal, the vagus gives off a branch, the course of which is entirely intracranial. Its ganglion is at its origin and gradually tapers off along its course. It passes vertically upward in the perimeningeal tissue, at first just behind the superior sinus of the labyrinth, but it gradually passes somewhat medial to it and in front of the supracerebral perilabyrinthine canal. In the perimeningeal tissue under the cranial roof it divides into several branches. I have not traced all these to their destinations; one contributes to the nerve supply of the

lateral-line enlargement which occupies the temporal foramen; another appears to go to the commissural lateral-line canal; probably others contribute to the plexus formed by the ramus recurrens facialis.

Two occipital nerves leave the skull through the upright portion of the exoccipital bone.

DISCUSSION

The relations of the bony elements of the skull to each other and to the precoelomic diverticulum of the swimbladder and to the membranous labyrinth which have been described in this paper are essentially the same for all the other American clupeoid species which I have examined. They also seem to correspond to those of the European species in which the details of the ear-swimbladder relation have been investigated. Certain of the more conspicuous features in which other American species differ from P. pseudoharengus may now be briefly summarized.

In general, differences in skulls of the other species seem dependent chiefly upon differences in the degree of ossification. The lateral cartilage plate is more or less completely ossified in the adult skulls of the other American species; in Brevoortia and Alosa, about the only remains of this structure is that part which contains the anterior semicircular canal. In these forms also the hook-like process of bone from the epiotic around the upper end of the anterior semicircular canal forms a complete ring.

On the external surface of the skull of Pomolobus mediocris, the posterior osseous capsule is only slightly visible; in Brevoortia tyrannis the surface of this capsule is completely covered with bone except for an area in the epiotic fossa so small as to be visible only with a microscope; in Alosa sapidissima the surface of this capsule is entirely obliterated by the development of bone. In most of these species, also, the bone development in the anterior cranial floor is much more extensive than in P. pseudoharengus. The lateral parts of the basisphenoid bone are thickened into massive processes and the sharply projecting edge

of bone which separates the orbital from the ventral surface of the prootic is greatly developed (fig. 2). As a result of these differences in the relative bone development in this region, the third and fifth nerves of B. tyrannis and of A. sapidissima appear to exit from the skull through canals instead of mere foramina as is the case in P. pseudoharengus.

The morphology of the relation of the swimbladder diverticulum to the cranium is a difficult problem. The suggestion of de Beaufort has been discussed above. Tysowski has developed an elaborate theory, the factual basis of which seems to be the relation of the septum in the anterior bony capsule to the bone to which it is attached. This septum, being in intimate connection with the bone of the capsule—its structure, in fact, continuous with it—and being covered by a reflection of periosteum from the bone surface, is (according to Tysowski's view) morphologically a part of the bone itself.

These facts persuaded Tysowski that the swimbladder diverticulum does not pierce the floor of the skull and lie next to the labyrinth in a cavity hollowed out in the prootic bone, but that the septum is a part of the cranial floor, and the swimbladder vesicle in the chamber below it is as much outside the skull as in other forms. Similarly the wall of the bony capsule is not a simple structure, but is of double origin, the part above the septum developing differently than the part below. He considers that the floor of the skull in the basisphenoid region gives off a process dorsally, which bends over medially and so forms the upper chamber which he says is comparable with the saccular cavity. The inferior chamber is formed similarly by a lateral process (presumably from the basisphenoid bone) which bends ventrally to enclose the anterior membranous vesicle of the swimbladder.

Tysowski's theory outlined above presents an interesting hypothesis in that the ear-swimbladder mechanism in the Clupeoids can easily be correlated with the simpler, more primitive type. With this theory as a basis, we might assume a nearly complete series of transition stages from the primitive relation of apposition (as in the case of the ear and swimbladder of Sparidae

and Gadidae) through forms like Hyodon and Notopterus, up to the highly elaborated mechanism in Clupeidae. But, unfortunately, Tysowski's morphological conceptions are quite inconsistent with well-known facts regarding the bony structure of the clupeoid skull, concerning which there is no essential disagreement on the part of other investigators.

In support of his theory, he uses the following argument.

> Auf Grund meiner Befunde würde es mir schwer fallen, die Bullae osseae anteriores als ein Erzeugnis der Prootica anzusehen, da ich Prootica, ähnlich wie Opisthotica, infolge der breiteren Gestaltung des Basioccipitale und Basisphenoideum mehr nach aussen liegend, wie gewöhnlich mit der vorderen und äusseren Ampulle und dem äusseren Bogengang in Beziehung treten sehe, übrigens lässt auch die starke Ausbildung des Corpus basisphenoidei vermüten, dass er von den Knochenlamellen der Prootic nicht überbrückt sein kann und die kapselartigen Gebilde eher ihm den Prootic angehören.

The relation of these bones to each other and the location of the bony capsules I have demonstrated to be quite different from the relations as Tysowski conceives them. That the anterior osseus capsule, in its entirety, is a part of the prootic bone in the adult cannot be questioned. It has been so described by all writers on the subject; my own preparations also demonstrate it. The general relations of the fifth and seventh nerve complex to the capsule and to the bone in which it lies are the same as the relation of these nerves to the prootic in the typical teleost cranium.

In investigating these relations I made use of dried preparations of the skull, as well as freshly cleaned specimens cleared in xylol, some of which are stained with alizarin, to bring out the cartilage lines of articulation. Specimens prepared by the latter method were particularly satisfactory when examined under the binocular microscope by transmitted light. The lines of demarkation of the bony elements were so clearly visible as to leave no room for doubt as to their identity. My results agree with the descriptions of the clupeoid skull as given by previous workers on the subject and are also consistent with the osteological relations of other teleostean groups as described by Sagemehl ('91), Allis ('97), Brooks ('84), and others. In view of these considerations, it

cannot be true that the two chambers of the capsule are formed by processes of the basisphenoid bone, as Tysowski has assumed, and therefore his whole morphological theory falls to pieces. This conclusion is substantiated by de Beaufort's observations on the embryology of the precoelomic diverticulum. His figures show that the anterior membranous vesicle actually develops inside of the chondrocranium immediately under the membranous labyrinth. This agrees with my previous published statements, regarding the developing stages of Stolephorus mitchilli (Tracy, '11).

It is another question to explain the anterior bony capsule as "ein Erzeugnis der Prootica" in view of de Beaufort's observation that the bony capsule is formed by a process of bone development from the connective tissue surrounding the membranous vesicle.

Tysowski questioned Ridewood's identification of the so-called 'posterior wings of the parasphenoid' as a part of the parasphenoid bone. Relative to this, it is pertinent to refer to one of the most characteristic morphological features of the teleostean skull, viz., that the parasphenoid wings form the walls of all except the upper part of the eye-muscle canal. The parasphenoid wings in Clupeoids conform to this relation. It can easily be seen in dissections, and also in cross-sections of the skull, that the rectus eye muscles arise along the whole medial surface of these wings as far back as their extreme posterior end. There is nothing to indicate that these wings differ in any essential respect from the corresponding bones in other fishes, except in their unusual ventral and posterior extension.[1]

Tysowski's denial of the existence of the auditory foramen (which is described in all previous papers on the clupeoid skull) is also probably without substantial basis. It is true that on careful dissection a thin scale can be demonstrated over this foramen, but it is transparent and does not resemble bone in

[1] Tysowski compares the wings of the parasphenoid bone with the processus pharyngealis in Ostariophysae. Sagemehl ('91, p. 516) has suggested that the processus pharyngealis is formed by the ossification of a ligament passing from the posterior end of the base of the skull around the aorta to the swimbladder.

appearance; furthermore, examination of sections of the skull in this region show a dense membrane which apparently is not true bone. What this membrane may be morphologically can only be determined by a developmental study of this region of the cranium, but the existence of the auditory foramen in the adult can hardly be denied.

The lateral recess has heretofore received scant attention. This is all the more remarkable when we consider that by means of this recess the exterior is brought into direct relation through the lateral-line canals with the fluid under the utriculus and so with the fluid through the whole system of the perilabyrinthine canals. These relations were first noted by Breschet, whose descriptions and figures show them somewhat vaguely. Most of the writers since have also referred to them, but only incidently and briefly. The work of Ridewood ('04 a) indicates that the lateral recess is a characteristic feature of the Clupeoid skull. He briefly refers to the cavity in Engraulis encrasicholus. "The lateral temporal groove is broad and shallow. Removal of its floor exposes a fairly large cavity opening laterally by two apertures. . . . This cavity is roofed by the frontal and is bounded in front by the postfrontal, prootic and squamosal, behind by the squamosal and below mainly by the prootic." In Coilia nasus, he describes the recess in similar terms and refers to the prootic bulla as projecting "upward into a lateral vacuity of doubtful homology." He states that the squamosal bulla (posterior bony capsule) is just visible in the hinder part of this cavity. This description corresponds to the relations of this cavity as described in this paper. Ridewood, however, did not mention the recess in connection with the other species which he studied. Matthews appears to have completely overlooked it. De Beaufort merely quotes Ridewood's statement.

The relations of the lateral recess are such that changes in pressure as the fish swims from one water level to another may be transmitted directly from the outside by way of the lateral-line canals and the loose tissue in the lateral recess to the fluid in the perilabyrinthine canals. Changes in hydrostatic pressure are thus conveyed directly to the walls of the utriculus. The struc-

ture of the macula acustica utriculi and its functional significance in connection with these mechanical relations will be discussed more fully in the paper to follow.

SUMMARY

1. The precoelomic diverticulum of the swimbladder divides into two branches which are conducted in a cartilage tube to the exoccipital bone on each side of the skull.

2. In canals in the bones at the base of the skull the diverticulum bifurcates; one branch passes laterally to the pterotic bone where it expands to form the posterior membranous vesicle contained in a spherical bony capsule; the other branch passes to the prootic bone where it forms the anterior membranous vesicle also surrounded by a bony capsule.

3. The osteological structure of the clupeoid cranium conforms to the type of the lower teleosts. Specialized features of the clupeoid cranium are:

a. The deep saccular recess partially covered by a triangular plate of the exoccipital bone.

b. The auditory foramen between the basioccipital, pterotic, and prootic bones.

c. The temporal foramen between the frontal, parietal, epiotic, and pterotic bones, occupied by a bay-like expansion of a lateral-line canal.

d. The lateral recess between the sphenotic, pterotic, prootic bones and the lateral wing of the frontal bone.

e. The falciform process of the prootic bones.

f. The great ventral and posterior extension of the wings of the parasphenoid bone.

g. The spherical osseus capsule in the prootic bone which communicates with the cavum cranii through a slit-like transverse fenestra.

h. The spherical osseus capsule in the pterotic bone.

i. The fusiform bulla in the exoccipital bone.

j. The inner wall of the lateral-line canals belonging to the lateral wing of the frontal bone is absent; the canals therefore lie in the loose tissue of the lateral recess.

4. The lateral recess communicates with the exterior through the lateral-line canals, and with the cavum cranni through the arch under the falciform process of the prootic bone. Hence there exists a free channel for the transmission of changes in water pressure from the outside to the fluid in the perilabyrinthine spaces.

5. The cranial nerves, from the third to the tenth, correspond in the general course and distribution of their branches to the nerves as described for Menidia. The branches of the trigeminofacial nerve complex make their exit from the skull in close relation to the surface of the prootic bony capsule. There is an intracranial plexus of nerves in the perimeningeal tissue supplied by recurrent branches of the seventh and tenth nerves. These recurrent branches also supply the expansions of the lateral-line canals in the lateral recess and in the temporal foramen. From the utricular branch of the eighth nerve, three branches are given off to supply the three divisions of the macula acustica utriculi.

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PLATES

ABBREVIATIONS

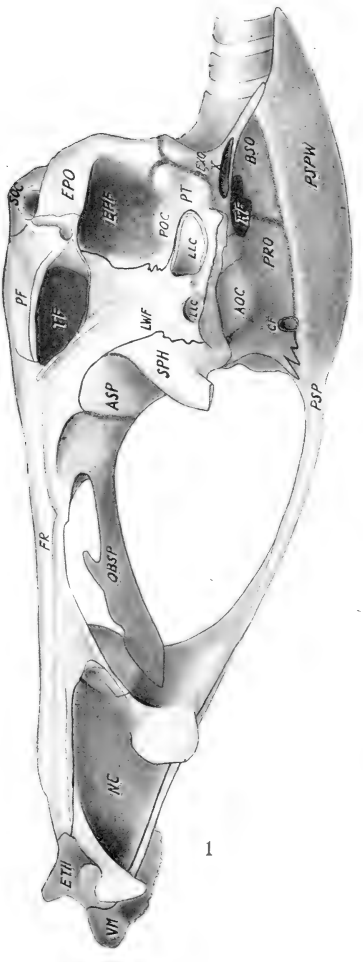
A, aorta
AF, auditory foramen
AMV, anterior membranous vesicle
AOC, anterior osseous capsule
AP, aponeurosis
ASC, anterior semicircular canal
ASP, alisphenoid bone
BR, brain
BSO, basioccipital bone
BSP, basisphenoid bone
CC, cartilage canal of the swimbladder diverticulum
CF, carotid foramen
CSD, canal of the swimbladder diverticulum
DC, opening of canal for swimbladder diverticulum
EPF, epiotic fossa
EPO, epiotic bone
ETH, ethmoid bone
EXO, exoccipital bone
F, fenestra
F III, foramen for oculomotor nerve
F V, foramen for trigeminofacial complex
FB, fusiform bulla
FCC, foramen for cartilage canal
FHM, foramen for hyomandibular nerve
FP, falciform process
FR, frontal bone
FSP, foramen for sympathetic and pretrematic VII
GF, ganglionic fossa
GL V, ganglion V nerve
GL VII, ganglion VII nerve
HMA, hyomandibular articulation
HY, hypophysis
HYF, hypophysial foramen
ILF, inferior lamina of the falciform process of prootic
K, kidney
LLC, lateral line canal
LPC, lateral cartilage plate
LR, lateral recess
LSC, lateral semicircular canal

LSCA, ampulla of lateral semicircular canal
LW, lateral wing of prootic
LWF, lateral wing of frontal
M, muscle
MPRO, mesial plate of prootic
NC, nasal cartilage
OBSP, orbitosphenoid
ON 1, first occipital nerve
ON 2, second occipital nerve
OPF, optic foramen
OPS, ramus ophthalmicus superficialis
OPT, opisthotic bone
PF, parietal bone
PMV, posterior membranous vesicle
PN, branch of VII nerve to ampulla of posterior semicircular canal
POC, posterior osseous capsule
PRO, prootic bone
PS, perilabyrinthine tissue space
PSC, supracerebral perilabyrinthine canal
PSD, posterior semicircular canal
PSDA, ampulla of posterior semicircular canal
PSP, parasphenoid bone
PSPW, posterior wings of parasphenoid
PT, pterotic bone
R VII, recurrent ramus of VII nerve
RE, rectus eye muscles
RHM, ramus hyomandibularis
RMM, ramus maxillomandibularis
RP VII, ramus palatinus of VII nerve
RPT, ramus pretrematicus
RSY, ramus sympatheticus
S, sacculus
SB, swimbladder
SBP, synchondrosis between basioccipital and prootic
SC, septum of anterior osseous capsule
SCA, prootic portion of saccular cavity
SCP, subcerebral plate of perilabyrinthine tissue
SLF, superior lamina of falciform process of prootic
SN, saccular division of VIII nerve
SOC, supraoccipital bone
SOL, supraorbital lateral line canal
SPH, sphenotic bone
SR, saccular recess
SS, superior sinus of utriculus
SSC, saccular subcerebral canal
TF, temporal foramen
TPEXO, triangular plate of exoccipital
VM, vomer
VT, vertebra
VBSO, ventral plate of basioccipital
VPRO, ventral plate of prootic
U, utriculus
USC, utricular subcerebral canal
III, oculomotor nerve
IV, trochlear nerve
V, trigeminal nerve
VI, abducens nerve
VIII, acoustic nerve
IX, glossopharyneal nerve
X, vagus nerve
*, foramen for ramus palatinus VII
x, foramen for hyomandibular nerve

PLATE 1

EXPLANATION OF FIGURES

1 Lateral view of skull of Pomolobus pseudoharengus. × 4.5.
2 Ventrolateral view of posterior part of skull of Alosa sapidissima. × 2.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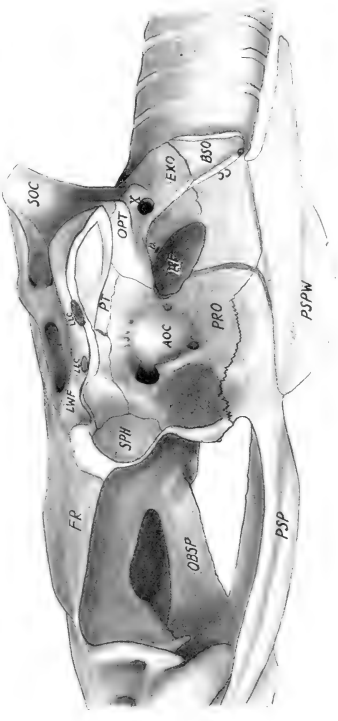
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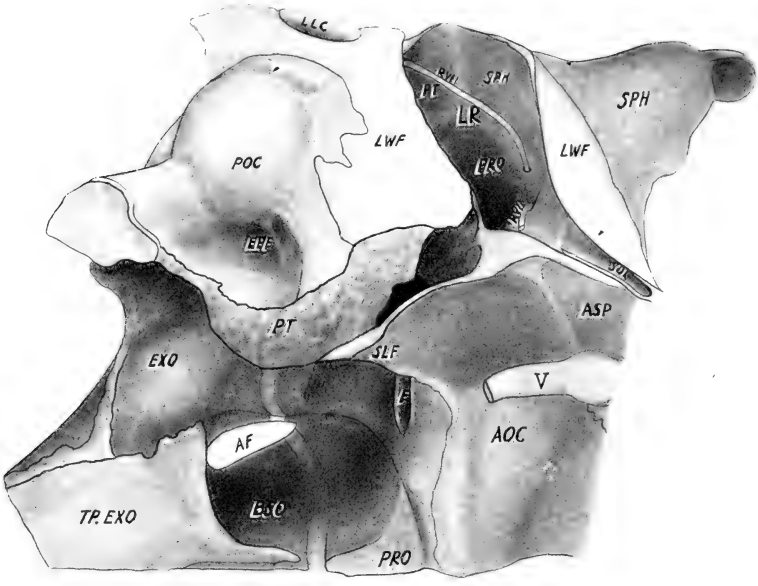
PLATE 2

EXPLANATION OF FIGURES

3 Bones of the floor and lateral wall of the skull viewed from above (Pomolobus pseudoharengus). A part of the lateral wing of the frontal bone has been removed to expose the lateral recess; the vertical, posterior part of the exoccipital has been cut away to show the floor and side of the saccular recess. × 9.

4 Bones of the floor and lateral wall of the skull viewed from the median side (Pomolobus pseudoharengus). The articulating surface of the prootic bone (*PRO*) is in the medial plane; the lateral surface of the basioccipital is shown (*BSO*); the frontal and sphenotic bones are removed. × 9.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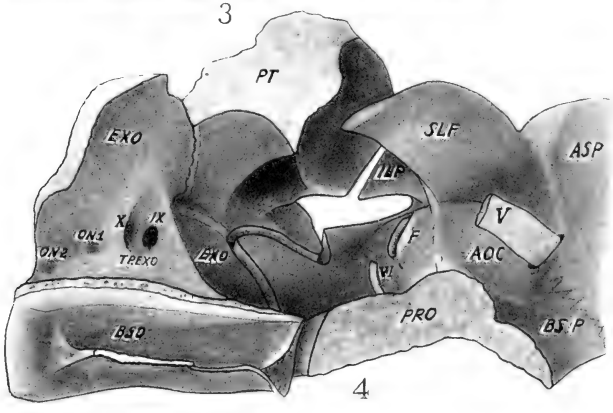
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PLATE 3

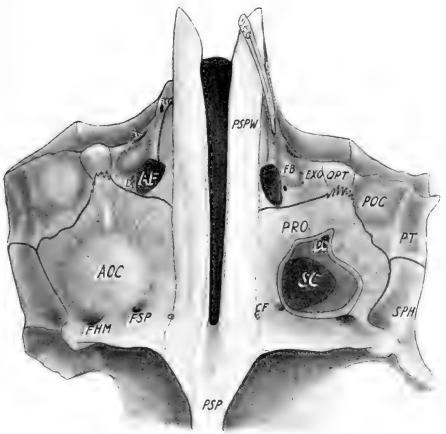
EXPLANATION OF FIGURES

5 Ventral surface of the cranium (Pomolobus pseudoharengus). On the right half of the figure the cartilage canal is shown; the ventral segment of the prootic osseous capsule (*AOC*) has been cut away to expose the septum and opening of the canal for the precoelomic diverticulum. × 5.4.

6 The cerebral surface of the prootic bone (Pomolobus pseudoharengus). × 6.

7 The ventral surface of the middle of the skull (Pomolobus pseudoharengus). × 5.

8 Medial surface of the lateral half of the cranium, showing the relations of the lateral cartilage plate, the perilabyrinthine canals, the nerves, and the eye-muscle canal (Pomolobus pseudoharengus). × 5.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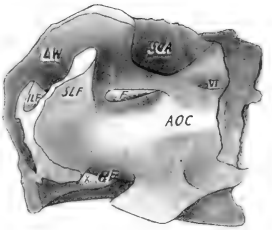
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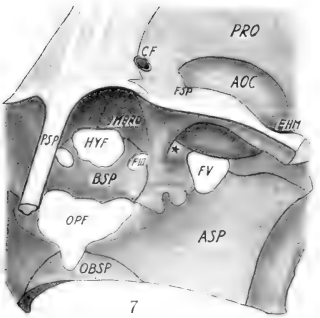
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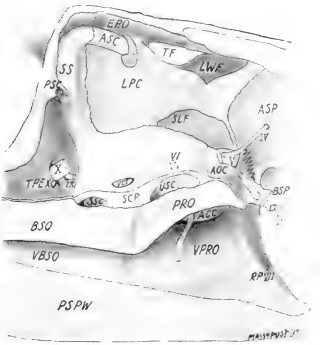
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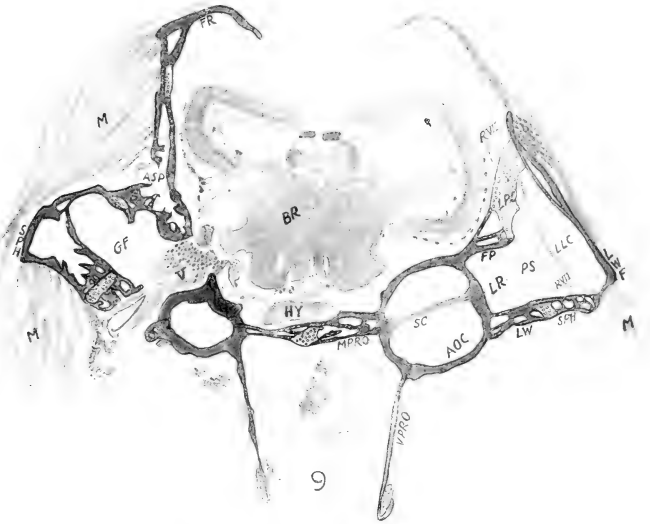
PLATE 4

EXPLANATION OF FIGURES

9 Cross-section through the head just behind the orbital region to show the bony structure of the side and base of the cranium. The section is oblique with their left side in advance; on the left side, the section passes through the ganglionic fossa, on the right through the lateral recess (Pomolobus pseudoharengus).

10 Cross-section through the head at the articulation between the prootic and basioccipital bones. On the left side the section passes through the front part of the posterior osseous capsule, on the right side, just in advance of that structure (Pomolobus pseudoharengus).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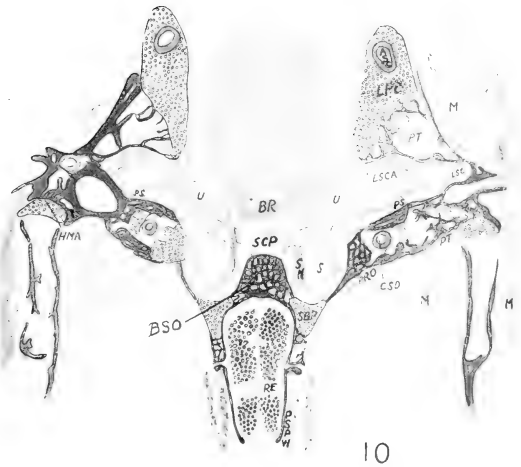
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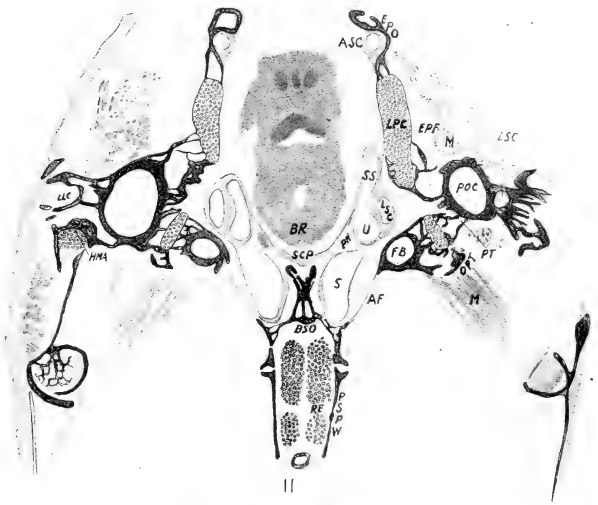
PLATE 5

EXPLANATION OF FIGURES

11 Cross-section of the head through the posterior osseous capsule and auditory foramen (Pomolobus pseudoharengus).

12 Cross-section through the occipital region of the head. The section is oblique with the right side in advance (Pomolobus pseudoharengus.)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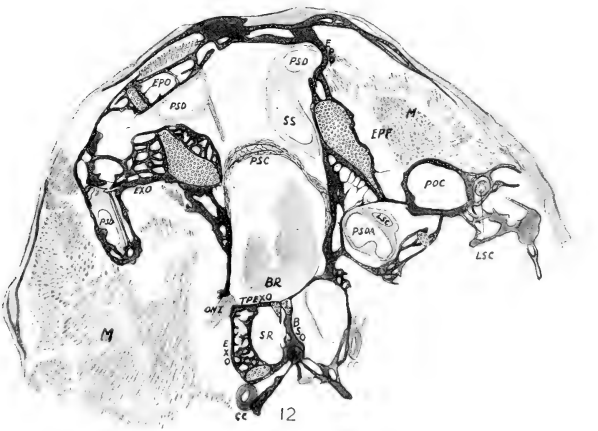
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12

Resumen por el autor, O. W. Hyman.
Universidad de Tennessee.

El desarrollo de Gelasimus después de abandonar el huevo.

El autor ha obtenido de los huevos de hembras ovígeras de Gelasimus pugilator, G. pugnax y G. minax, los primeros estados de zoés. Las larvas zoés de estas especies son tan semejantes entre sí que los esfuerzos para determinar la especie en los ejemplares capturados por medio de la sonda han sido estériles. Se criaron cuatro de los cinco estados de zoé por que pasan estos animales; las restantes formas fueron obtenidas mediante la sonda o en la playa, dejándolas sufrir una muda durante la observación. También ha estudiado y descrito los cambios que tienen lugar durante la metamorfosis de las zoés, megalops y el cangrejo jóven hasta el momento de la diferenciación sexual. Los apéndices abdominales del estado megalops son estructuras larvarias. Los apéndices abdominales del adulto son formaciones nuevas que aparecen al adoptar el animal la forma de cangrejo. Algunas de las costumbres de las hembras ovígeras, zoés, megalops y cangrejos jóvenes se han descrito incidentalmente.

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THE DEVELOPMENT OF GELASIMUS AFTER HATCHING

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TWELVE PLATES (EIGHTY-EIGHT FIGURES)

During the summers of 1915 and 1916 at the U. S. Fisheries Biological Station at Beaufort, North Carolina, while engaged in an endeavor to rear crustacean larvae under artificial conditions, I had an opportunity to study the habits and developmental stages of Gelasimus. While this study was only incidental to the experiments in hand, I found the material so abundant and other conditions so favorable that I have been enabled to review the development in considerable detail. During the progress of the study I have been aided greatly by the criticism and guidance of Dr. H. V. Wilson and Mr. W. P. Hay. The work done at Beaufort has been made a pleasure by the generous cooperation unfailingly extended by Mr. S. F. Hildebrand, director of the laboratory.

OCCURRENCE OF THE ADULTS

Of the many decapods at Beaufort, the three species of Gelasimus are perhaps the most numerous. Gelasimus pugilator, the common gray sand-fiddler or fiddler crab, is present almost everywhere, but is most abundant on the islands and shores where a sandy beach is exposed at low tide. Conditions are especially favorable if the beaches have a fringe of sedges which are in the water at high tide. The crabs find a ready refuge in these when frightened.

Gelasimus pugnax is not so abundant as G. pugilator, but is rather common. It is most often found in marshes, especially where there is a considerable estuary formed at high tide, but where the soft boggy marsh is exposed when the tide is out. One such

estuary leads north from Taylor's creek, a few hundred yards west from Lenoxville Point. This species is also abundant on the banks of the Beaufort end of the Inlet Canal from Pamlico to Beaufort.

The third species, Gelasimus minax, is not common. It seems to prefer higher ground bordering a marsh, and frequently occurs at some distance from salt water. It occurs along the banks of the Inlet Canal from Pamlico to Beaufort, and especially in one of the large estuaries on the Shackelford Banks east of the Mullet Pond.

METHODS OF STUDY

In studying the larval history of these forms, the first zoeae were hatched in the laboratory. The ovigerous females of G. pugnax and G. minax were secured readily by digging them from their burrows in the marshes. Egg-bearing females of G. pugilator at first were very hard to find. I have dug the crabs from their burrows for hours and searched the hundreds at the water's edge at ebb tide without finding a single ovigerous female. Quite by accident I discovered a method of securing all the eggs of this species to be desired. Landing on Horse Island just at dusk one evening, I noticed that a number of the fiddlers hurrying to their burrows were egg-bearing females. Investigation showed that a large percentage of the females carried eggs. Thereafter I had no difficulty in securing all the eggs I wished by going to some favorable spot at dusk during an ebb tide. The crabs were taken to the laboratory and kept in crystallization dishes partially filled with water, until the eggs hatched. Generally the eggs hatched within a few days. The approximate age of the embryos can be determined at a glance. When the eggs are newly laid they are a deep purple—almost black. As the embryo develops it becomes lighter and distinctly purple. It continues to lose its color, becoming a dirty gray when it is nearly ready to hatch. The eggs of all three species hatch soon after 7 P.M., that is, at about dusk. This fact probably explains the presence of the females at the water's edge at that time.

Rearing the larvae. During the summers of 1915 and 1916 I made many unsuccessful attempts to rear the larvae secured by hatching the eggs while they were still on the female. I carried one solitary specimen through three molts, several through two molts, and many through one molt, but the great majority of the zoeae died before their first molt. The only method by which I succeeded in carrying any beyond the first molt was as follows: Small floats, 6 inches by 8 inches by 2.5 inches, with sides of fine bolting cloth, were made. The zoeae were placed in these and these bolting-cloth floats then placed in crab floats for protection from the rippling of the surface water. The difficulty with this method was that debris rapidly collected on the float and clogged its meshes. The bolting cloth rotted quickly and it was difficult to recover the zoeae.

Larvae from the tow. The fifth zoea and the megalops and crab stages were not reared from the egg. The method of tracing the development through these stages was to collect specimens of some known stage from the tow and keep them in the laboratory until the next molt. The zoeal stages could rarely be kept alive through more than one molt. On the other hand, the megalops and crab stages could be kept alive indefinitely if they were fed and protected from the cannibalistic tendencies of their fellows.

The stages for study were obtained as follows. The first, second, and third zoeal stages were abundant in the surface tow from July 1st to September 15th. The tow might be taken anywhere in the harbor or outside. The stages could be separated with some difficulty and placed in glass bowls. The next molt generally occurred in a few days.

The fourth and fifth zoeal stages seldom appeared in the surface tow. These stages, however, could be obtained readily from the bottom tow. The method of towing on the bottom was as follows: A bolting-cloth sac was made to fit inside a small heavy, bottom dredge. This was then dragged carefully along with just enough rope to let it touch the bottom. Care must be exercised to keep it from digging. The only successful tows of this kind were taken over a sandy bottom. The best place was found to be along the southwest edge of Bird Island Shoal.

Megalops and crab stages. The specimens of megalops were taken most abundantly in surface tow from the ocean just outside the inlet. They were also taken in numbers from crevices in rotting boards exposed at low tide, under oyster shells and stones, or from the bark on pilings. The crab stages do not appear in the tow. They may be picked from the old boards, with the megalops stages, or obtained during ebb tide on a sandy beach (where they are very hard to see) or in the debris in the marshes. After the crab measures about 2 mm. across the carapace, it digs its own burrow and may then be collected by digging. All of the crab stages are found mingled with the adults.

HISTORY OF THE DEVELOPMENT

From the foregoing facts the following history of the development may be deduced with reasonable assurance. The egg-laden females remain hidden in their burrows during the day, probably because the egg mass retards their movements to such an extent as to endanger their existence. At dusk, when the eggs are ready for hatching, the females approach the water's edge and the eggs are hatched in the water. The larval skin with which the embryo is covered is shed in hatching.

The young zoea wobbles off on the surface of the water, being carried along largely by the tide. Its own efforts, however, serve to keep it at the surface, all the zoeal forms being positively phototropic. After about four days the first molt occurs. The second zoea behaves like the first—keeps itself near the surface of the water by the rapid beating of the maxillipeds and is swept along by the tides. After four or five days a second molt occurs. The third zoea is the form most rarely found in the tow. This indicates the possibility that it swims at an intermediate depth, the maxillipeds not being strong enough to sustain at the surface the increased weight of the body.

After the molt to the fourth zoeal form, the zoea sinks. It does not lie or crawl on the bottom, but is swept along by the current and at short intervals drives itself upward by the rapid beating of the maxillipeds. As soon as the maxillipeds cease beating it falls slowly to the bottom again on account of its

weight. After about a week the next molt occurs. The fifth zoea lives near the bottom like the fourth. It is still more disproportionately heavy and correspondingly clumsy. During the last day or so of this stage the animal is almost entirely at the mercy of the tide. The next molt occurs at the expiration of a week or ten days.

When the zoea molts to the megalops stage, its mode of life suddenly changes. The animal is now provided with powerful swimming organs, the pleopods, so situated as to be most efficient. Its chelae serve as an excellent means of securing its prey, which now consists, partly at least, of smaller crustaceans. Its organs of equilibration are suddenly well developed and its other sense organs are more nearly perfect. The animal rapidly ascends to the surface and darts swiftly about. The megalops stage probably lasts a long time, and there is only one such stage. Megalopa were kept in the laboratory as long as three weeks before molting to the crab stage. All of those that molted became crabs at the first molt. After swimming about at the surface for three or four weeks, the megalops seeks some protected place, such as the crevices in a rotting board near the shore, and there undergoes the molt to the first crab stage.

The young crab clings closely to its refuge or crawls about at the water's edge, especially among the exposed roots of sedges. It is very clumsy and very weak. At the end of three days it molts to the second crab stage. After four or five days a second molt occurs. After this molt the little crab runs about quite freely and may dig its first burrow. It now measures about 2 mm. across its carapace at its broadest point. Its mode of life from now on is like that of the adult.

IDENTIFICATION OF THE ZOEAE

The zoeal forms of Gelasimus may be identified readily. They have prominent anterior and dorsal spines, but have no lateral spines on the carapace. This distinguishes them at once from all the other common zoeae of the Beaufort region, except those of the two species of Sesarma. From these the zoeae of Gelasi-

mus may be distinguished in two ways: the length of the antenna does not equal that of the anterior spine in the case of Gelasimus, but does in Sesarma; the first maxillipeds of Sesarma have pigment spots at the proximal ends of the protopodites, while in Gelasimus the first maxillipeds have pigment spots at the distal ends of the protopodites.

The pigmentation of all the zoeal stages of Gelasimus is remarkably constant and serves as a ready means of establishing the identity of the form. The pigment spots are jet-black when contracted. In the expanded condition they vary in color, being black or olive or red-brown or orange or combinations of these colors. The distribution of the spots is as follows: on the carapace, a spot posterior to the base of the dorsal spine, a spot on each lateral flange of the carapace near its posterior angle, a median spot between the eyes, a large spot on the front of the base of the anterior spine, a spot between the bases of the first and second maxillae; on the appendages, a spot on the labrum, one on each mandible, and one on the distal border of the protopodite of each of the first maxillipeds; on the abdomen, a pair of dorso-lateral spots between the first and second segments, a pair of ventral spots on the second and third segments, and lateral spots on each side of the posterior borders of the fourth and fifth segments.

DISTINGUISHING CHARACTERISTICS OF THE ZOEAL STAGES

The first and second zoeal stages of the three species were obtained with certainty by hatching and rearing them in the laboratory. The distinctions between equivalent stages of the three species, however, all proved to be relative differences of such slight degree that I was never sure that I could separate certainly the specimens obtained from the tow. Some specimens had the characteristic broad-based, evenly tapering frontal spine of G. pugilator and others the slender constricted spine of G. pugnax, but many of them seemed intermediate, and I gave up the attempt to distinguish the species. The different developmental stages of the zoeae were easily distinguishable from each other, however, as is indicated in the descriptions below.

First zoeal stage of G. pugilator (figs. 1 and 2). These zoeae are relatively small (length from head between the eyes to tip of telson, 1 mm.). They swim by means of the first and second maxillipeds, and, so far as was observed, swim in only one direction, upward and slightly forward. When at rest the maxillipeds are habitually carried in the position shown in figure 1. In swimming, these are raised to the sides of the carapace and driven downward. When not swimming, the larva is nearly always actively writhing about, chiefly by lashing the abdomen. The first and second maxillae beat regularly and rapidly in such a way as to drive a current toward the mouth opening.

The carapace is slightly flattened from side to side. It bears the usual anterior and dorsal spines, but shows no traces of lateral spines. The anterior spine rises from the anterior margin of the carapace between the eyes and passes ventrally almost at right angles to the long axis of the body. It is about 0.2 mm. long, straight, smooth, and evenly tapering from a slightly swollen base. The dorsal spine arises in the mid-dorsal line posterior to the eyes and just above the heart. It is shorter than the anterior spine and curved posteriorly. There are constantly present a pair of setae which arise on each side of the carapace, anterior and lateral to the base of the dorsal spine. The lateral ventral borders of the carapace show the usual anterior and posterior lobes.

The eyes are sessile and immovable. The facets are clearly indicated, but are not perfectly marked on the surface. The antennule (fig. 20) is 0.07 mm. long and conical. From its tip arise two or three long olfactory hairs and one or two short, sharp-pointed setae. The antenna (fig. 28) is 0.11 mm. long and bisegmented. The proximal part of the basal segment is thick and cylindrical. At its distal end its inner half is produced into a stout serrated spine about twice as long as the proximal portion. The outer half of the tip of the basal portion bears the distal segment, which is small and cylindrical. From its tip arise two setae, one long, which seems to be a continuation of the segment, and a short outer one.

The mandible is short, stout, and unsegmented. Its edge has the usual teeth for tearing and grinding. The first maxilla (fig. 45) is bisegmented. The basal segment is bilobed and thickly lamelliform. The medial lobe bears one movable smooth spine on its median border, and, at its tip, three macerating spines. The lateral lobe bears similar spines arranged in two series. From the distal border of the lobe arise two or three strong spines, and from its inner face, near the border, arise three weaker spines. The distal segment is cylindrical and bears four tactile hairs at its tip.

The second maxilla (figure 54) is a lamellar appendage, its median border produced into four lobes and a hairy process extending laterally. The three median lobes represent the basal segment or segments. Of these, the most median bears two series of smooth spines. The middle and lateral lobes of the basal segment are each armed with three macerating spines at their tip and one on their inner surface near the tip. The fourth lobe represents the distal segment. It bears three tactile hairs at its tip. The outer plate represents the epipodite. It consists of a proximal lamelliform portion which bears four finely plumose hairs along its lateral border, and is produced posteriorly into a process which tapers to a blunt end. The process is covered with fine hairs over most of its suface.

The first maxilliped (fig. 62) is the best developed of the appendages at this time. It is 0.25 mm. long without its terminal hairs. It is composed of a basal portion, an endopodite, and exopodite. The basal portion is unsegmented, compressed, and of approximately uniform circumference. The endopodite, slender and slightly longer than the basal portion, is composed of five segments. The terminal segment bears three tactile hairs at its tip and a single plumose seta from its median superior surface. The exopodite is unsegmented, cylindrical, and about equal in length to the endopodite. It bears four long plumose hairs which are jointed near their middle. The length of the hairs is from 0.16 to 0.20 mm.

The second maxilliped (fig. 62) is like the first in all respects except its endopodite. This is much shorter and is trisegmented.

The two basal segments are very short, the terminal segment is like the terminal segment of the endopodite of the first maxilliped.

The abdomen is composed of five movable segments. Each of the first four is cylindrical and of approximately the same diameter. They increase slightly in length from the first to the fourth. The second, third, and fourth segments are produced backward and laterally into an angular process which slightly overlaps the next succeeding segment. The posterior border of each of these segments bears a median seta dorsally. The second segment bears a short blunt lateral spine which curves forward. It is so placed that its curvature serves as a groove into which the posterior border of the carapace fits. The third segment also bears a spine which curves backward on each side and is less conspicuous. The terminal segment represents the sixth abdominal segment fused with the telson. It is crescent-shaped with the horns elongated. The anus lies on the ventral surface of this segment. It is surrounded by very tumid, movable lips. which may form a protuberance as in figure 1. From the median surface of each horn near its base, three setae arise which are plumose with short stout hairs. The length of the segment with its horns is 0.22 mm.

First zoeal stage of G. pugnax (figs. 3 and 4). The first zoea of G. pugnax differs from that of G. pugilator only in size. It is smaller in all dimensions. The anterior and dorsal spines are shorter and slenderer. Otherwise there is the most absolute identity in pigmentation and conformation of the appendages—even to the number and kind of hairs found on each.

First zoeal stage of G. minax (figs. 5 and 6). The first zoea of G. minax is distinguishable from that of G. pugnax with the greatest difficulty. It is slightly smaller, but shows the same slender spines of the carapace.

The first zoeal stage of Gelasimus (figs. 1 to 6) is most readily distinguished by the four plumose hairs of the exopodites of the maxillipeds. The caudal portion of the scaphognathite is a single elongated conical process thickly beset with fine hairs.

The second zoea (figs. 7 and 8) has increased in length to 1.175 mm. The eyes are on stalks and are slightly movable. The

lateral hairs on the scaphognathite are now five and the posterior conical portion is tripartite distally (fig. 56). The exopodites of the first and second maxillipeds become bisegmented and the number of hairs at their tips is increased to six.

The third zoea (figs. 9 and 19) has increased in length to 1.5 mm. The eyes are freely movable. A broad, low tubercle appears between the spine of the basal segment of the antenna and the base of the distal segment (fig. 30). This is the anlage of the flagellum of the permanent antenna. The distal portion of the first maxilla (fig. 48) has two segments and has become separated from the basal portion by a joint. In the second maxilla (fig. 57) the scaphognathite now bears six hairs along the lateral border of its anterior portion. The exopodites of the first and second maxillipeds bear eight hairs. The third maxillipeds are clearly distinguishable for the first time as minute buds just behind and somewhat median to the second pair. The buds of four pairs of periopods are distinguishable and those of the cheliped are somewhat larger and show an identation at their tips indicating the position of the chela. Blunt protuberances from the ventral surfaces of the second to sixth abdominal segments are the beginnings of the pleopods. On the telson (fig. 84) are two minute spines medial to those earlier present. There is a deep groove between the sixth abdominal segment and the telson, but no joint has yet developed.

The fourth zoea (fig. 11) has increased in length to 2 mm. The antennule has increased in size somewhat and a lateral hair is present near its tip (fig. 24). The tubercle of the antenna is greatly enlarged and the former distal segment now appears as a lateral appendage (fig. 32). The second maxilla shows several changes (fig. 58). The palp is separated from the basal portion by a joint and the median lobes are more pronounced. The scaphognathite appears as a single plate and bears eighteen hairs along its lateral border. The first and second maxillipeds have nine or ten hairs at the tips of their exopodites. The bud of the third maxilliped is notched at its tip, indicating a division into two rami. The buds of all the periopods appear, and those of the chelipeds are clearly bifurcated. The buds of the pleopods

have become more prominent. The border of the carapace bears a series of straining hairs which serve to keep foreign particles from getting under it.

The fifth zoea (figs. 12 and 13) has increased in size to a length of 2.25 mm. The antennule (fig. 25) shows the following changes: a rather deep constriction divides the distal portion from the slightly enlarged basal portion; on the distal portion the number of hairs of the second series is increased from the single one to three. When this stage is nearing its molting time, the distal portion shows indistinctly two or three constrictions where the joints of the next stage will appear. In the antenna (fig. 33) the flagellum is bisegmented and is marked off by a joint. As the time for the next molt approaches, the flagellum shows indications of about twelve constrictions which mark out the joints of the next stage. The first maxilla shows a few minor, but interesting changes (fig. 50). There is developed on its lateral border a rounded low prominence which bears a single sparsely plumose stout hair similar to those found on the coxopodites of the maxillipeds at the base of the epipodite. Between the palp and the epipodital prominence is a peculiar, densely plumose hair similar in structure to the hair on the distal segments of the endopodites of the first and second zoeal maxillipeds and to the so-called 'auditory hair' of Mysis. The exopodites of the first and second maxillipeds bear ten hairs (figs. 66 and 67). The endopodite of the second maxilliped has grown considerably larger. The third maxillipeds and the periopods are finger-shaped appendages, and two or three small buds dorsal to their bases are the early gills. The pleopods are also finger-shaped and show indications of division into protopodite, exopodite, and endopodite, although the endopodite is exceedingly minute. The telson bears four pairs of plumose spines. During the last day or two of this stage a number of changes are noticeable in preparation for the next molt. The soft part is withdrawn from the dorsal spine, until it is entirely empty, and from the anterior spine until it fills the basal fourth only. The exopodites of the maxillipeds are shrunken away from their coverings, thus accounting for the sluggishness of the larva at this time. All the joints of the periopods are differentiated.

Description of the Megalops (figs. 14 and 15). When the fifth zoea molts to the form of the megalops, a profound change occurs in many of the parts. The contours of the cephalothorax and the abdomen are both changed. Throughout the zoeal stages the cephalothorax is flattened from side to side. In the megalops it is flattened dorso-ventrally. The abdomen is cylindrical in the zoea and now becomes flattened dorso-ventrally also. The changes in many of the appendages are still more striking. The animal suddenly becomes well equipped for an active predatory existence. The sensory structures of the antennule and the antenna are practically in the adult condition. The chelae are efficient structures for securing the prey and the maxillipeds are transformed into masticatory organs. The pleopods are now developed into powerful swimming organs and the animal darts swiftly about.

The antennule (fig. 26) is now composed of a large basal portion and a terminal process of four segments. The basal segment bears the statocyst which can be distinguished through its walls. The ultimate and penultimate segments bear from five to seven olfactory hairs each. The antennule has now reached what is practically the adult condition. The antenna has undergone a striking change (fig. 34). The zoeal lateral spine and lateral segment are absent. The flagellum is composed of eight small cylindrical segments and is borne at the tip of a basal portion of three larger segments. The antepenultimate segment of the flagellum bears four or five long tactile hairs and the terminal segment two or three.

The mandible (figs. 40 and 41) has reached practically the adult condition, as it now bears a three-jointed palp. The first maxilla (fig. 51) shows few changes. Its basal median lobe bears more spines and is enlarged. The joints of the palp are obscure and its segments somewhat shriveled. The hairs on the lateral border of the basal portion have the same form as the hairs on the epipodites of some of the appendages posterior to it. The second maxilla (fig. 60) has undergone changes similar to those of the first. The palp has lost its hairs and joint and appears as a smooth lobe of the basal portion. The scaphognathite is larger and has more hairs along its border.

The changes in the maxillipeds are profound (figs. 68, 70, and 74). The first and second pairs are transformed from swimming organs and all three pairs become functional as mouth parts. The first and third pairs bear well-developed epipodites, and each of the second pair bears a tiny bud on its lateral surface—the beginning of an epipodite and a gill. In all these appendages the proximal segment of the exopodite is elongated and slender, while the distal segment is small and is carried at right angles to the basal segment. It bears three or four weak, slender, plumose hairs. The endopodite of the first pair is twisted so that the lateral edge of its distal portion becomes median. It bears only a few small hairs. The endopodites of the second and third pairs are composed of four segments, are stout, and bear numerous macerating spines.

The gills of the megalops are four on each side—a pleurobranch between the third maxilliped and the cheliped, two podobranchs on the cheliped, and one podobranch on the second periopod.

The chelipeds are large and functional as pincers. The second, third, and fourth periopods are long and slender with somewhat hooked extremities. They may be used in crawling, but are used chiefly for clinging to some protecting cover. The fifth periopod seems to be useless. It is small and has several long hairs at its tip, and is carried folded over the back of the carapace.

The pleopods (fig. 79) are large, well-developed swimming organs. Each is composed of a basal segment bearing an exopodite and an endopodite. The exopodite is a flattened lobe bearing from seven to fourteen swimming hairs around its border. The endopodite (fig. 81) is small, bears no hairs, but has three shriveled, curled processes at its tip which may represent atrophied hairs.

The first crab stage (fig. 16). When the megalops molts to the first crab stage, the cephalothorax is slightly altered, becoming broader and more depressed. The abdomen shows a great change. It is permanently flexed under the thorax into a groove in which it fits, and its appendages, the pleopods, are shriveled and hidden under it.

The antennules and antennae show only slight changes. The mandible and first maxilla are very slightly altered also, but the second maxilla (fig. 61) is changed both in shape and in the relative size of its parts. The scaphognathite has increased in size; the two basal lobes are larger and are partly constricted from the coxal segment; the palp is relatively smaller and is reduced to an inconspicuous lobe of the lateral basal lobe.

The maxillipeds (figs. 69, 71, and 75) show changes, but none so marked as in the previous molt. In the first, the change is largely an increase in size and apparent strength. The endopodite has changed shape and is now a trisegmented, flattened appendage with the middle segment twisted or folded on itself. All its segments are hairy. The basipodite and coxopodite appear as prominent rounded lobes medially, but fuse into an unjointed mass laterally. Each lobe has a spiny border. The epipodite is larger and bears scattered, slender, barbed hairs. The second maxilliped is not greatly changed. The proximal segment of the endopodite is relatively larger and bears a row of stout spines along its median border. The tubercle of the future gill and epipodite shows a differentiation into minute lobes. The change in the third maxilliped is largely confined to the endopodite, which is relatively larger, due to an increase in size of the proximal two segments. The number of hairs is increased on all segments, but especially on the lateral portions of the protopodite and the proximal half of the epipodite.

The chelae are further developed. Both the carpus and the dactyl end in rounded, spoon-shaped points. Both chelae are identical. The fifth periopods are adapted for walking and clinging. They are small, but have the usual five segments of the periopods.

The most striking change among the appendages occurs in the pleopods. These are no longer the well-formed, powerful swimming organs of the megalops, but are smaller, hairless, and shriveled. They are hidden between the abdomen and the thorax.

The second crab stage (fig. 17). After the next molt the crab has increased in size from 1.35 mm. long and 1.25 mm. broad to

1.5 mm. long and 1.9 mm. broad, thus showing a relative broadening of the carapace. The lateral borders of the carapace are still lobulated and beaded, but not so prominently as before. The only changes of note in the appendages are the assumption of adult form by the antenna (fig. 36) and further reduction of the pleopods. The antenna is now made up of a large basal segment and a flagellum. The proximal two joints of the flagellum are distinct, but the others are reduced to surface constrictions. The pleopods are distinguishable as minute, shriveled appendages on the second, third, fourth, and fifth abdominal segments, but are absent from the sixth. The abdomen has begun to broaden by the development of lateral flanges.

The third crab stage (fig. 18). There is no pronounced change at the next molt except in the pleopods. They may be entirely absent in this stage or may be present on the first to the fifth segments as buds so minute as to be indistinguishable under magnification less than five hundred diameters. Those on the second segment may be larger than the others. The abdomen has become broader.

Beginning of sexual differentiation. After the next molt the young crab attains a width of carapace of 3 mm. This stage shows the beginning of sexual differentiation; in males one chela is slightly larger than the other. Abdominal appendages of a second series make their appearance. These develop into the genital appendages of the adult. In male specimens appendages are present as minute buds on the first and second segments. In the female buds are distinguishable with difficulty on all the the segments from the second to the fifth.

Description of a 4-mm. crab. When the crab reaches a width of 4 mm. across the carapace, the sexual differentiation is pronounced and other important changes have occurred. The carapace now has the adult shape with straight sides. Numerous very brushy hairs have appeared on its anterior surface below the orbits. The abdomen is still further flattened and its segments seem to be more or less completely fused except at their lateral borders. The telson, however, is freely movable, being joined to the rest of the abdomen by a membranous joint. The whole

abdomen fits tightly into its groove on the thorax. Its lateral borders are beset with numerous straining hairs. The hairs around the telson are numerous and brushy.

The eyes (fig. 19) have now reached their adult condition. They are bisegmented and the terminal segment bears the compound eye facets over its distal and lateral faces. The eyes are carried erected over the carapace in this stage, but may be lowered into their imperfect orbits for protection.

No changes have occurred in the first five pairs of appendages except slight changes in the relative sizes of some of their parts and a multiplication of the hairs on each.

The first and third maxillipeds show no change of importance. They are more hairy and some of their hairs have developed into so-called 'comb hairs.' On the second maxilliped (fig. 73) the gill and the epidodite are now developed, although both are quite small. The gills present in this stage are as follows: a podobranch on the second maxilliped; two arthrobranchs or pleurobranchs between the third maxilliped and the cheliped; two pleurobranchs at the base of the cheliped, and one pleurobranch at the base of the second period.

In the female the chelipeds are not differentiated, but both remain small with spoon-shaped extremities (fig. 78). In the male, one of the claws is considerably enlarged, is thicker, and is adapted to cutting and pinching (fig. 77). The spoon-shaped chelae are especially adapted for scooping up the fine sand from which the animals get their food.

The abdominal appendages are now modified to form sexual organs. In the male, the appendages of the first and second abdominal appendages only are present. Each consists of two segments. The appendage of the first segment is composed of a rather broad basal portion and a rod-like distal segment. The distal segment is grooved along its median border. The appendage of the second segment is much smaller than that of the first, but has the same enlarged basal segment and rod-like distal segment. The distal segment, however, is cylindrical. In the female, appendages appear on the second to the fifth segments. Each is composed of a basal portion and two rami. Both rami

are cylindrical and the endopodite is bisegmented. None of the parts are separated by distinct joints as yet. The appendages of the second segment are the largest, the others becoming progressively smaller from before backward.

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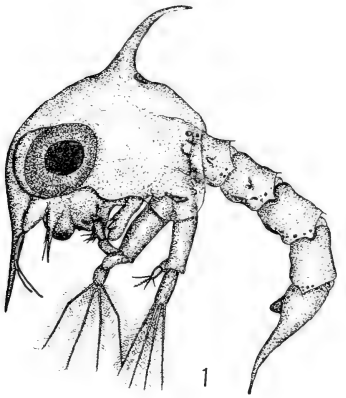
PLATE 1

EXPLANATION OF FIGURES

1 First zoea, G. pugilator, lateral view
2 First zoea, G. pugilator, front view.
3 First zoea, G. pugnax, lateral view.
4 First zoea, G. pugnax, front view.
5 First zoea, G. minax, lateral view.
6 First zoea, G. minax, front view.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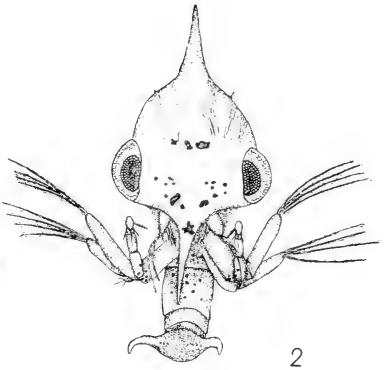
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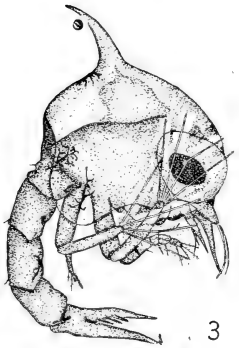
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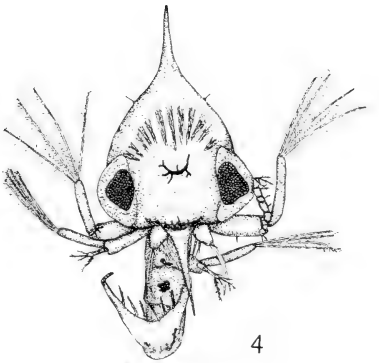
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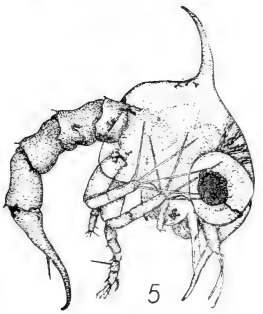
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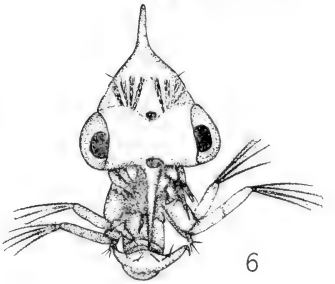
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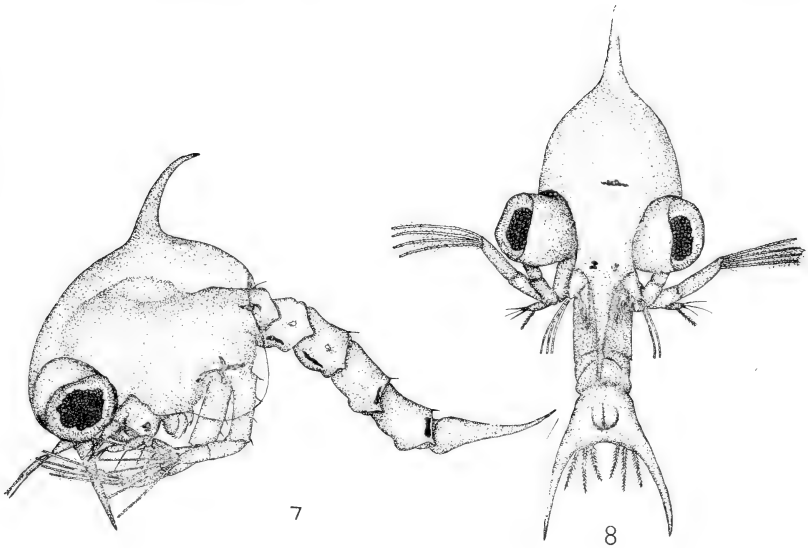
PLATE 2

EXPLANATION OF FIGURES

7 Second zoea, G. pugnax, lateral view.
8 Second zoea, G. pugnax, front view.
9 Third zoea, G. pugilator, lateral view.
10 Third zoea, G. pugilator, front view.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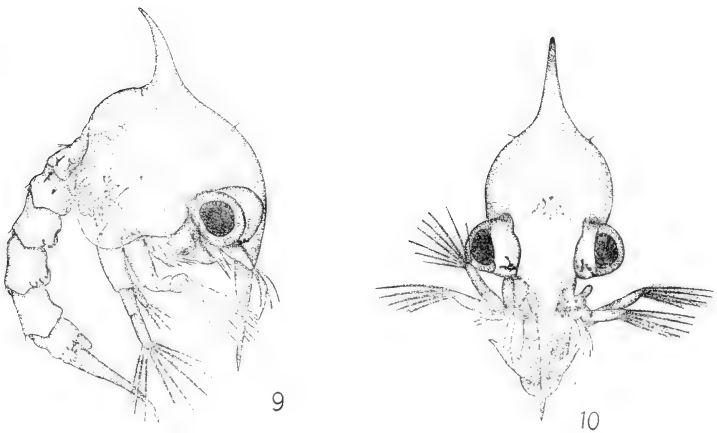
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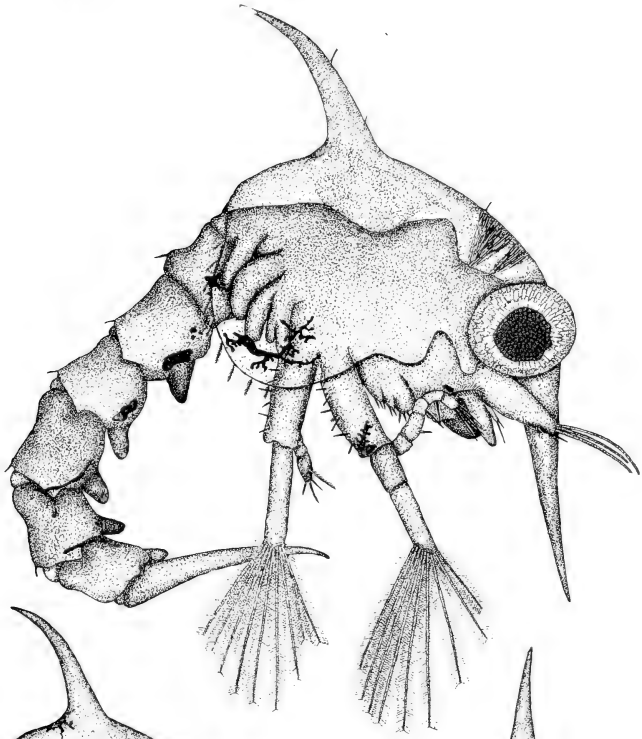
PLATE 3

EXPLANATION OF FIGURES

11 Fourth zoea, G. pugilator, lateral view.
12 Fifth zoea, G. pugilator, lateral view.
13 Fifth zoea, G. pugilator, front view.



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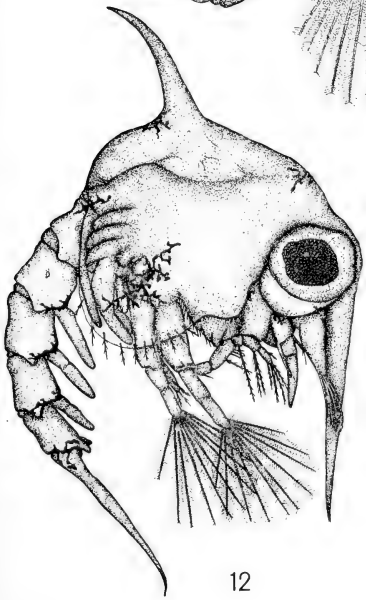

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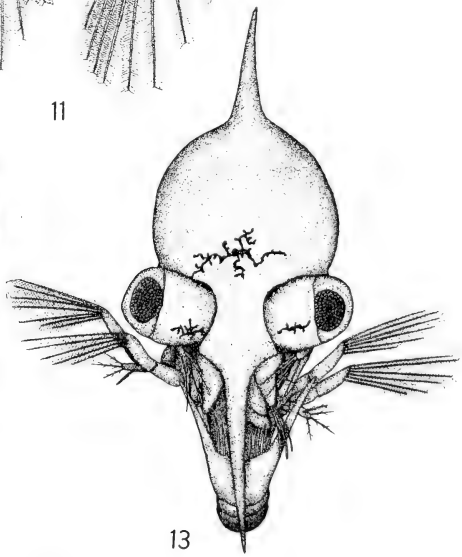

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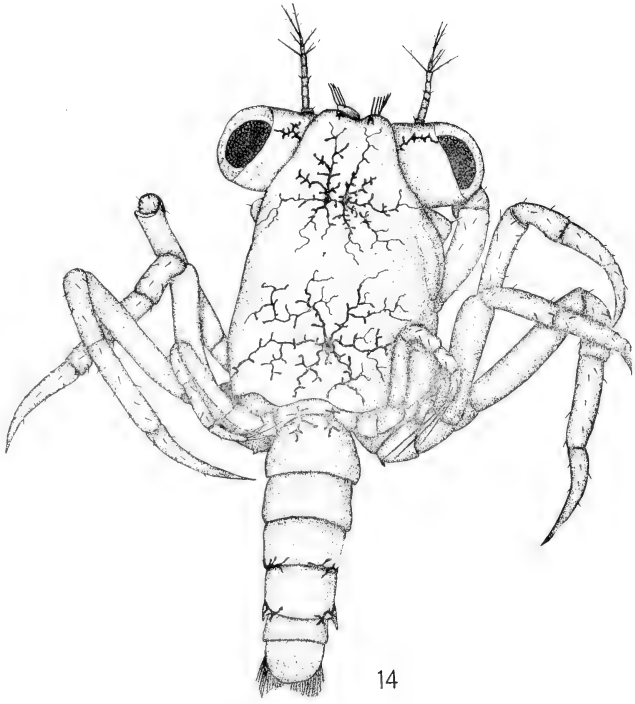
PLATE 4

EXPLANATION OF FIGURES

14 Megalops, G. pugilator, dorsal view.
15 Megalops, G. pugilator, ventral view.



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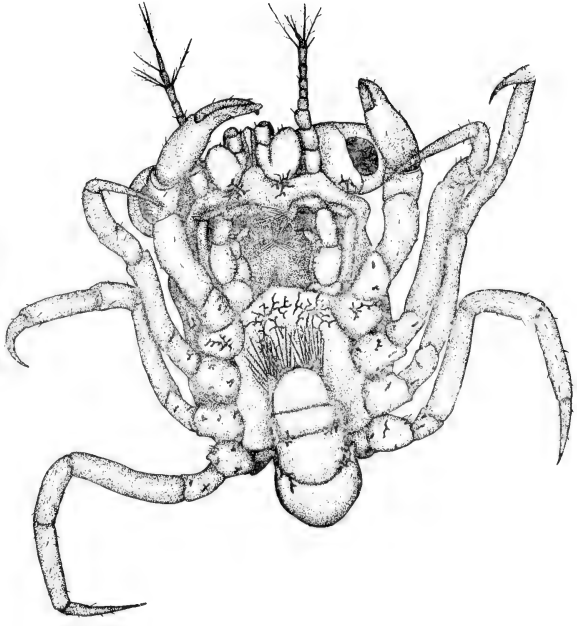

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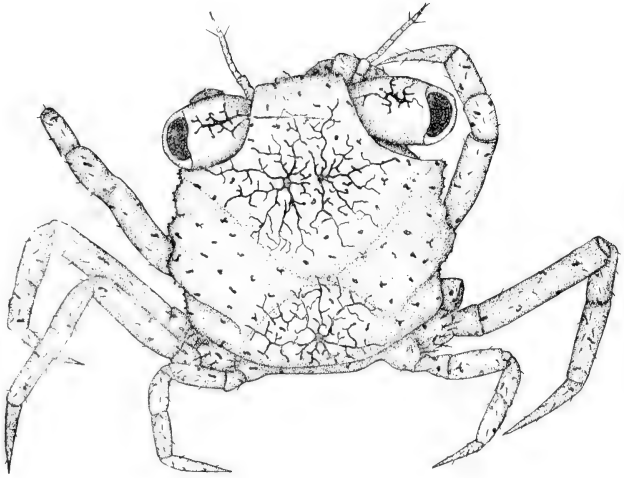
PLATE 5

EXPLANATION OF FIGURES

16 First crab stage, G. pugilator
17 Second crab stage, G. pugilator.
18 Third crab stage, G. pugilator.



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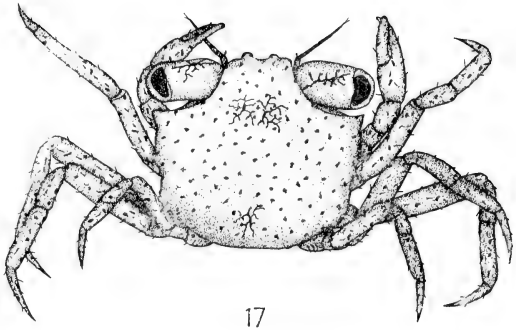

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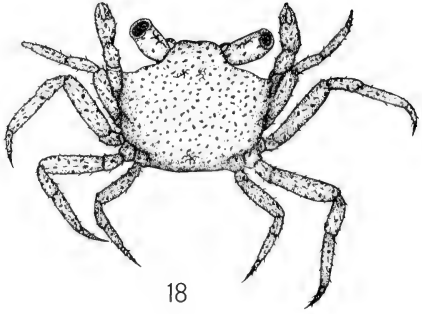

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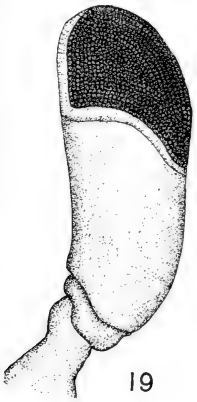
PLATE 6

EXPLANATION OF FIGURES

19 Eye, 4-mm. crab, G. pugilator.
20 Antennule, first zoea, G. pugilator.
21 Antennule, first zoea, G. minax.
22 Antennule, second zoea, G. pugnax.
23 Antennule, third zoea, G. pugilator.
24 Antennule, fourth zoea, G. pugilator.
25 Antennule, fifth zoea, G. pugilator.
26 Antennule, megalops.
27 Antennule, first crab stage.
28 Antenna, first zoea, G. pugilator.
29 Antenna, first zoea, G. minax.
30 Antenna, second zoea, G. pugnax.
31 Antenna, third zoea, G. pugilator.
32 Antenna, fourth zoea, G. pugilator.
33 Antenna, fifth zoea, G. pugilator.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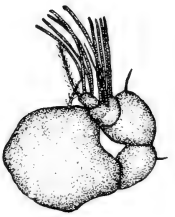
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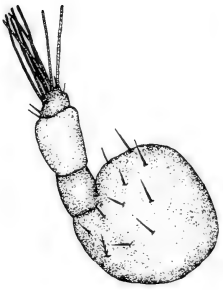
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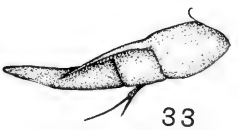
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PLATE 7

EXPLANATION OF FIGURES

34 Antenna, megalops.
35 Antenna, first crab stage.
36 Antenna, second crab stage.
37 Mandibles, first zoea, G. minax.
38 Mandible, third zoea, G. pugilator.
39 Mandible, fifth zoea, G. pugilator.
40 Mandible, anterior surface, with palp pulled off, megalops.
41 Mandible, from above, megalops.
42 Mandible, posterior surface, first crab stage.
43 Mandible, anterior surface, first crab stage.
44 Tip of mandible, posterior surface, second crab stage.
45 First maxilla, first zoea, G. pugilator.
46 First maxilla, first zoea, G. minax.
47 First maxilla, second zoea, G. pugnax.
48 First maxilla, third zoea, G. pugilator.
49 First maxilla, fourth zoea, G. pugilator.
50 First maxilla, fifth zoea, G. pugilator.

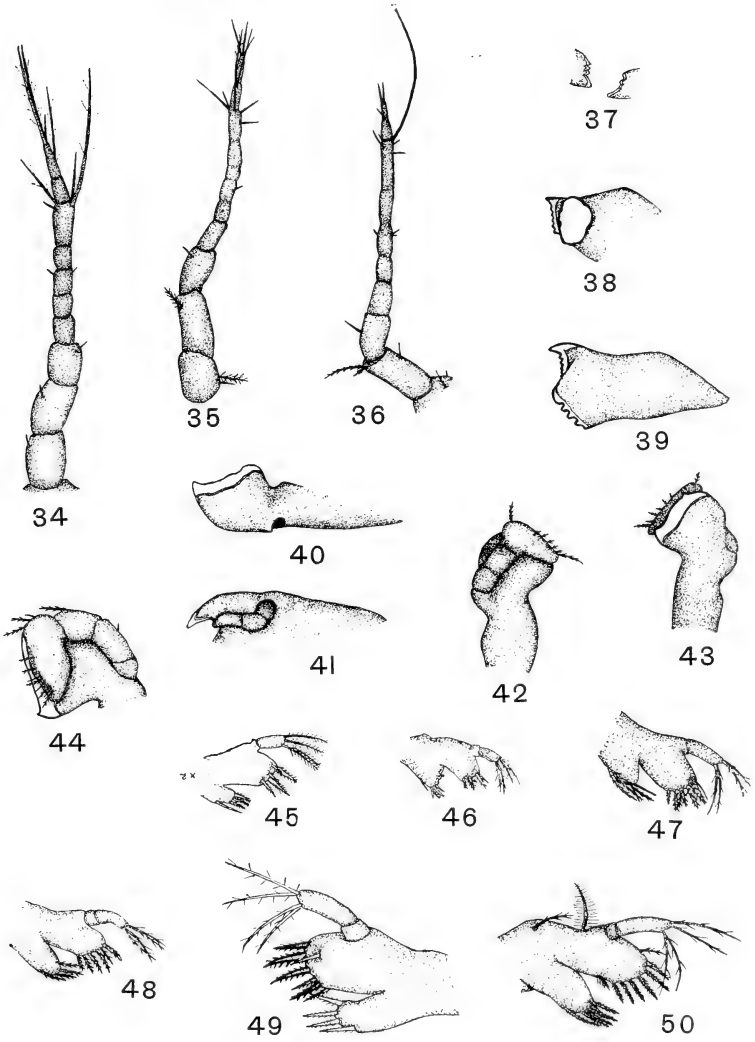
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PLATE 8

EXPLANATION OF FIGURES

51 First maxilla, megalops.
52 First maxilla, first crab stage.
53 First maxilla, 4-mm crab stage.
54 Second maxilla, first zoea, G. pugilator.
55 Second maxilla, first zoea, G. minax.
56 Second maxilla, second zoea, G. pugnax.
57 Second maxilla, third zoea, G. pugilator.
58 Second maxilla, fourth zoea, G. pugilator.
59 Second maxilla, fifth zoea, G. pugilator.
60 Second maxilla, megalops.
61 Second maxilla, first crab stage.


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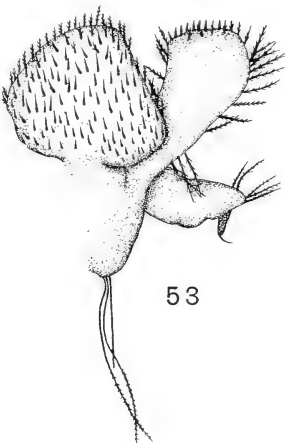
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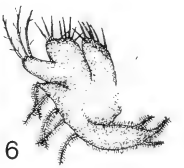
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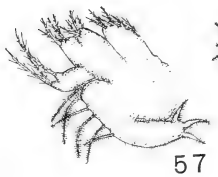
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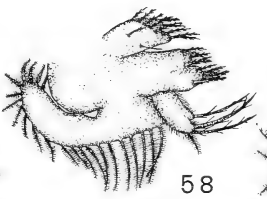
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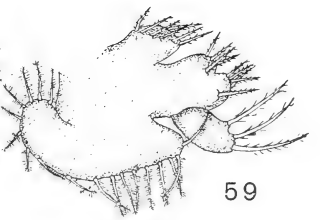
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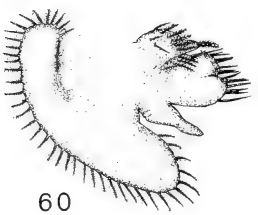
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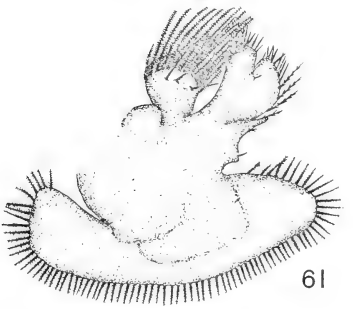
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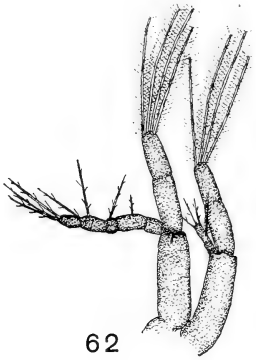
PLATE 9

EXPLANATION OF FIGURES

62 First and second maxillipeds, first zoea, G. pugnax.
63 First maxilliped, first zoea, G. minax.
64 Second maxilliped, first zoea, G. minax.
65 First and second maxillipeds, third zoea, G. pugilator.
66 First maxilliped, fifth zoea, G. pugilator.
67 Second maxilliped, fifth zoea, G. pugilator.



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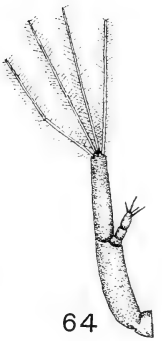

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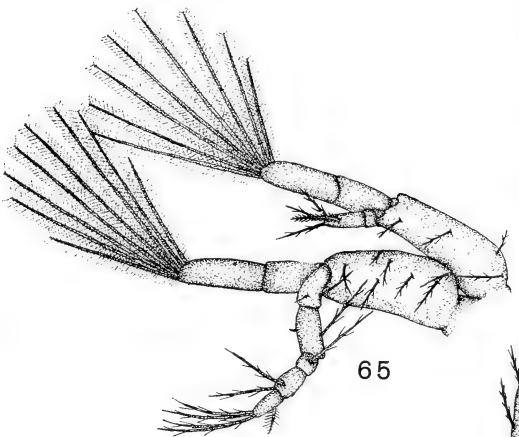

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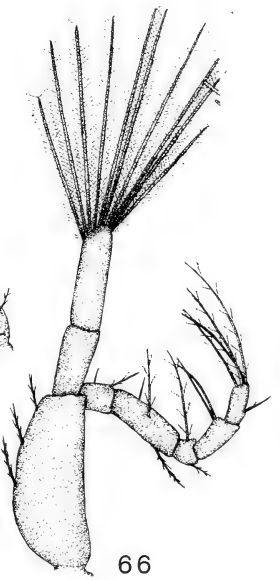

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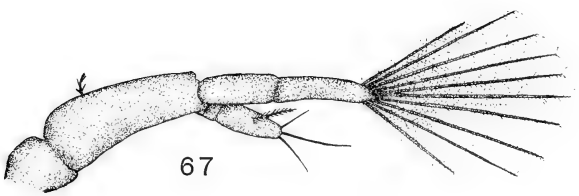

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PLATE 10

EXPLANATION OF FIGURES

68 First maxilliped, megalops, without coxal segment.
69 First maxilliped, first crab stage, without coxal segment.
70 Second maxilliped, megalops.
71 Second maxilliped, first crab stage.
72 Tip of comb hair.
73 Second maxilliped, 4-mm. crab stage.


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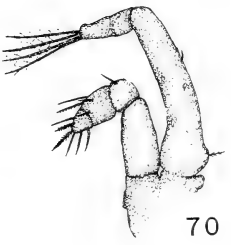
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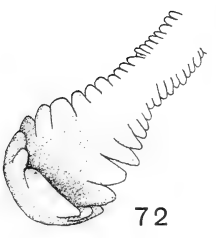
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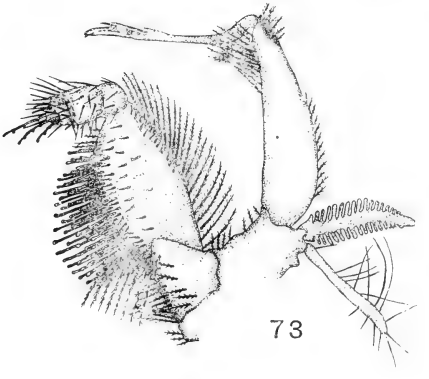
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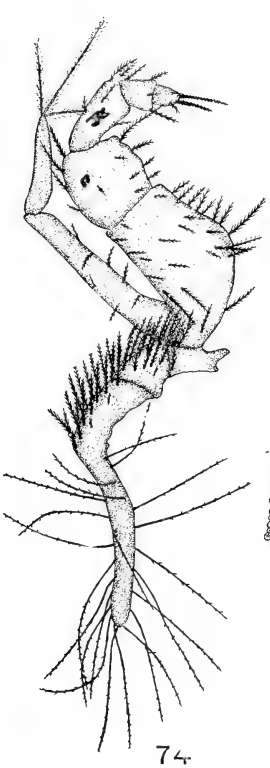
PLATE 11

EXPLANATION OF FIGURES

74 Third maxilliped, megalops.
75 Third maxilliped, first crab stage.
76 Left chela, megalops.
77 Left chela, 4-mm. crab stage.
78 Right chela, 4-mm. crab stage.
79 Second pleopod, megalops.
80 Fifth pleopod, megalops.



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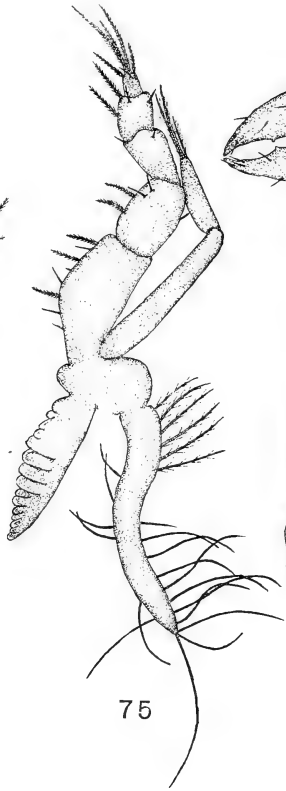

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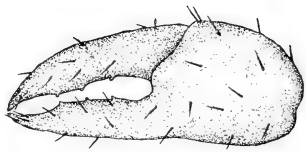

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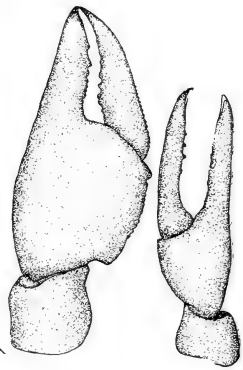

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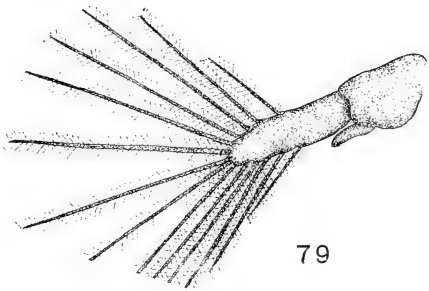

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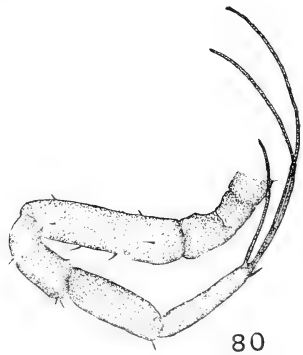

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PLATE 12

EXPLANATION OF FIGURES

81 Endopodite, second pleopod, megalops.
82 Telson, first zoea, G. pugilator.
83 Telson, first zoea, G. minax.
84 Telson, third zoea, G. pugilator.
85 Telson, fourth zoea, G. pugilator.
86 Telson, fifth zoea, G. pugilator.
87 Telson, megalops.
88 Telson, first crab stage.

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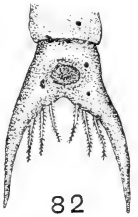

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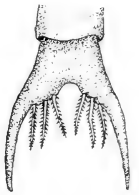

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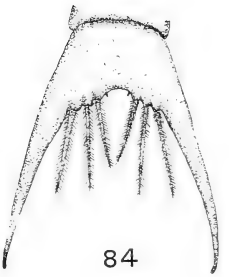

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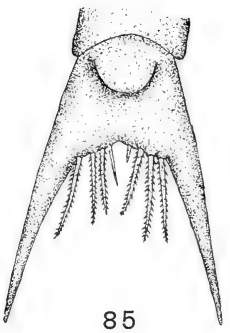

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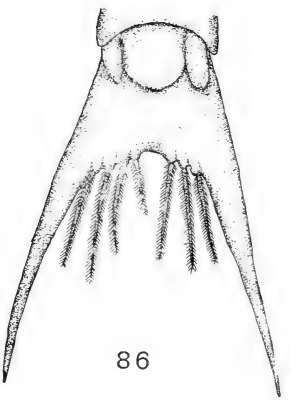

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Resumen por la autora, Louise Smith.
Colegio Smith, Northampton, Massachusettts.

El aparato hiobranquial de Spelerpes bislineatus.

La autora ha hecho un estudio morfológico del aparato hiobranquial, esqueleto y músculos, en diferentes estados del desarrollo de Spelerpes bislineatus comparándole con las mismas partes de otros urodelos. Los métodos empleados han consistido en cortes seriados, preparaciones teñidas en masa con azul de metileno y disecciones. El aparato larvario está caracterizado por una firmeza y rigidez considerables, en parte debidas a a presencia de una placa branquial formada por la fusión de los primeros ceratobranquiales con el segundo basibranquial. La presencia de esta placa es un fenómeno universal en las larvas de los Salamándridos, pero su existencia no se ha reconocido generalmente. En el adulto, los músculos y cartílagos de la lengua, que es libre y boletiforme, han sido objeto de una investigación especial en el presente trabajo. El aparato en conjunto es muy delicado y delgado y capaz de una gran complejidad de movimientos, estando adaptado al modo de respiración y a la captura de las presas. La transición de la estructura de este aparato desde la larva al adulto ha sido seguida durante la metamorfosis, habiendo trazado la autora un cuadro normal. La transformación del aparato hiobranquial tiene lugar no por una mera absorción de las partes destinadas a desaparecer y el simple cambio de posición de otras, sino por un proceso complicado que implica la degeneración y pérdida de ciertas partes a consecuencia de fagocitosis, y la formación de nuevos tejidos en la posición ocupada por el aparato del adulto.

Translation by José F. Nonidez
Carnegie Institution of Washington

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THE HYOBRANCHIAL APPARATUS OF SPELERPES BISLINEATUS

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Department of Zoology, Smith College, Northampton, Massachusetts

FORTY-SEVEN FIGURES

CONTENTS

INTRODUCTION

1. Purpose and scope

The study of the hyobranchial apparatus of Spelerpes bislineatus[1] was undertaken in the hope that its results might prove of some significance to the broader investigation which Mrs. I. W. Wilder is carrying on in regard to the metamorphosis of this animal. As the whole process of metamorphosis is a series of adaptations to the transition from a wholly aquatic to a semi-terrestrial life, and, as especially important adaptations come in

[1] In their Checklist ('17) Stejneger and Barbour have replaced the generic name Spelerpes by Eurycea, a Rafinesquian name. As Spelerpes has been universally accepted in morphological literature for nearly seventy years, there seems to be no necessity for adopting the change here.

response to the necessity for a changed mode of respiration and a changed diet at that time, an anatomical description of the apparatus associated with these phenomena may be of interest.

It is of course the hyobranchial skeleton which supports the external gills of the larva and its muscles that move them in the many varied and characteristic ways. The first basibranchial and ceratohyals also support the very primitive larval tongue, which is merely a fold in the mucous membrane filled in with connective tissue and scarcely more pronounced than the lateral oblique folds in the floor of the mouth that are an adaption to the movement of the gill arches. Whatever motion this larval tongue, so like that of the fishes, is capable of, is also brought about by the hyobranchial muscles.

With the loss of the external gills at metamorphosis, the function of the visceral skeleton as a support for the organs of respiration is minimized, though its muscles still bring about the characteristic movements of the pharynx that accompany buccopharyngeal respiration. Its main purpose now seems to be to support and move the very specialized tongue which has developed for capturing and disposing of the more rapidly swimming, jumping and flying prey of the adult. The adult tongue is a mushroom-shaped organ capable of a great variety of motion. Its stalk is formed by the basibranchial cartilage and the pair of abdominohyoideus muscles, and its very glandular disk is supported by the tip of this basal piece and associated cartilages and regulated by fibers of the hyoglossal muscle (fig. 2). It is capable of being withdrawn wholly into the mouth, with the disk parallel to the stalk, and the mucous membrane of the latter folded back like a semi-inverted glove finger; of being extended, with the disk 3 or more millimeters beyond the mandible and moved through an angle of 90°, so that it is perpendicular to the stalk, or of assuming any of the innumerable positions between these two.

The purpose of this paper is to give the anatomical basis for further investigation of the physiological phenomena related to the functions of respiration and capturing and swallowing of prey, and more particularly for understanding these activities during the period of transformation.

In the carrying out of this plan I shall attempt to give a complete description of the anatomy of the hyobranchial skeleton and of its muscles, without reference to their innervation, in the larva and in the adult, with some reference to the morphology of the parts, and shall then try to trace the method of development of the larval apparatus into that of the adult, through the period of metamorphosis. Correlation of the facts found with physiological observations, in the main, fall outside the scope of the present paper.

2. Material and technique

I have used a large number of specimens of Spelerpes bislineatus, larval, metamorphic, and adult, many of which had been previously observed for external physiological phenomena in the living state. I prepared about twenty-five or thirty of these for study of the skeleton by Van Wijhe's methylen-blue method for cartilage. This method was particularly good for the larvae after killing in 10 per cent formalin, but did not give quite such successful results with the adults and metamorphic specimens. Figures 4, 5, 17, 18, and 28 are drawn from such preparations.

I could dissect the adults very successfully under the binocular microscope, especially if stained first in methylen-blue. Figures 19, 20, and 21 are based upon such dissections. For the more detailed work, however, serial sections were the surest method. Several larvae of about 20 to 24 mm. were sectioned for the typical larval condition, and several adults for comparison with my dissections. For the metamorphic phenomena I made series of some eleven specimens, which I afterward found fell into about four distinct stages. My sections are 15 and 20 μ thick and mainly transverse, with a few horizontal and sagittal series for comparison. Delafield's haematoxylin after the picric acid of the decalcifying fluid was the most useful stain.

Reconstructions were made with millimeter paper (the drawing of the larval muscles (fig. 6) is made from one of these) and certain details I reconstructed with wax plates. But for the main part I relied on rough reconstruction in plastiline, and on

comparison with methylen-blue preparations, dissections, and other series of this work.

For comparison, dissections were made of Necturus and Cryptobranchus; methylen-blue preparations of Diemyctylus viridescens, Amblystoma opacum, and Spelerpes ruber; dissections under the binocular of Spelerpes ruber, adult Amblystoma opacum, and adult Salamandra maculosa; serial sections of a Salamandra larva, Necturus embryos, a small Cryptobranchus, an Axolotl, Typhlomolge rathbuni, and of several stages of Desmognathus fusca were studied.

At this point, I wish to express my sincere thanks to Dr. H. H. Wilder for his valuable advice and criticism; to Mrs. Wilder for many helpful suggestions and for the use of her specimens and notes on metamorphosis, and to Mr. E. R. Dunn for material from his amphibian collection.

LARVAL CONDITION

1. Hyobranchial skeleton

The hyobranchial apparatus in the larva of Spelerpes bislineatus consists of the hyoid and three branchial arches (figs. 4 and 5). The individual arches are not clearly defined, however, as in the lower fishes, for in some instances certain cartilages are missing and in others the exact morphology of parts is doubtful. The apparatus consists, in the main, of paired cartilages which are hung, directly or indirectly, on a single median basal piece. This basal piece or first basibranchial, a rather small, more or less cylindrical cartilage which lies well forward in the midline of the floor of the pharynx, is thus of considerable physiological importance. Anterior and lateral to it, is the pair of ceratohyals, the sole remnants of the hyoid arch. The ceratohyal is a long, heavy cartilage, the largest in the whole apparatus. At its proximal end, it articulates with the anterior end of the first basibranchial and projects posterolaterally to be suspended from the skull by connective tissue at its distal end.

Ventral to the posterior half of the first basibranchial and also median, is a somewhat triangular, dorsally concave branchial

plate with thickened lateral edges. It is notched at its apex, and fits around the middle of the shaft of the first basibranchial to form a more or less freely movable articulation. Posteriorly, as shown in cross-section (figs. 9 and 10), it seems to hold the basibranchial and proximal ends of the second epibranchials within its concavity, as in a trough. Its lateral thickenings are prolonged into short, stout shafts which undoubtedly represent the distal ends of the first ceratobranchials and with which the long, heavy first epibranchials are articulated. In addition to the lateral projections is a median, posterior one (probably the second basibranchial,[2] ending in the widening of the cartilage which, in adult life, becomes ossified to form the os thyreoideum. Thus, it is evident that the plate is, in reality, a fusion of three cartilages, the first ceratobranchials and the second basibranchial. This makes it difficult to determine just what elements are actually involved in the first branchial arch.

The second branchial arch, however, quite plainly consists of a pair of cerato- and a pair of epibranchials. The second ceratobranchial is a short, slender cartilage which articulates, proximally, with the posterior end of the first basibranchial and, distally, with the proximal end of the long, delicate second epibranchial.

The third branchial arch is represented by a delicate epibranchial alone, which articulates proximally with the median edge of the second epibranchial; the fourth arch, merely by a raphé in the muscles as in Necturus, and the fifth, represented in the lunged forms by the arytaenoids, has disappeared entirely.

The branchial plate is the most significant feature of the visceral skeleton of the larva. Although it is apparently a universal phenomenon among larval Salamandrids[3] and is unmistakably

[2] In calling this cartilage the second basibranchial I follow Wiedersheim, whose nomenclature of the hyobranchial skeleton I have used throughout this paper. As it is not absolutely proved that this is morphologically the second basibranchial, many zoologists, noticeably followers of Gegenbaur, prefer to be non-committal and call it 'copula.'

[3] In retaining the term 'Salamandrid' as a convenient method of designating the Urodeles that undergo complete metamorphosis, I am aware that recent systematists no longer make this distinction.

shown by a study of both sections and preparations in toto (figs. 4, 5, 9, and 10), it has escaped the notice of most authors, who picture the first ceratobranchials and the second basibranchial as quite separate from each other and articulating with the first basibranchial in the same plane with the second ceratobranchials. In fact, in the literature I find only two references to the plate. Gaupp ('06, p. 705) incidentally mentions that in the visceral skeleton of Triton taeniatus larva, "Der ventrale Teil hat die Form einer breiten dreieckigen Platte, die hinten in einen langen medianen Fortsatz, den Copulastiel (=second basibranchial), auslaüft," and pictures it plainly (p. 706, fig. 35). Mrs. Wilder has carefully worked out the anatomy of the hyobranchial skeleton in Desmognathus fusca and shows, without any shadow of a doubt, that ". . . . the second basibranchial cartilage . . . . during larval life forms one continuous chondrification with the first pair of ceratobranchials" ('13, p. 320). This she pictures clearly (p. 321, fig. 25 (a)) by a drawing of a methylen-blue preparation.

Parker ('80) and Drüner ('02) just miss showing it for Salamandra maculosa. The former pictures a section a little posterior to the position of the actual continuity of the cartilages and notes that the first ceratobranchials lie ventral to the second, and the latter comes even nearer the truth, for he not only says that the first ceratobranchials "nicht in derselben Frontalebene wie die Hypobranchiale 2 (= second ceratobranchials) sondern ventral verschoben liegt" (p. 471), but also shows a ventral piece (Taf. 25, fig. 3) which corresponds to the second basibranchial plus the median portion of the plate, and with which he makes the first ceratobranchials articulate. If he had not shown this articulation, which I am convinced by study of a series of cross-sections, does not exist, he, too, would have pictured the plate.

I have stated that the plate is probably a universal phenomenon among the larval Salamandrids only, because it is not present even in very young stages of Necturus or Cryptobranchus or in Siren lacertina,[4] but it is present in important representatives of

[4] In Siren a transitional form may be present, as here the first ceratobranchials and second basibranchials are slightly ventral to the rest of the apparatus (cf. H. H. Wilder, '91, pl. 29, fig. 7).

each of the three families of Urodeles that undergo complete metamorphosis. Thus, among the Plethodontidae, Desmognathus fusca, Typhlomolge rathbuni,[5] and Spelerpes ruber and bislineatus; among the Salamandridae, Triton taeniatus and cristatus, Salamandra maculosa, and Diemyctylus viridescens, and among the Amblystomidae, Amblystoma opacum and punctatum, and the axolotl, all have this fusion of the cartilages. Had time and material permitted my carrying this investigation further, I have no doubt I should have found similar results throughout the group, for the plate seems to have an important physiological function in these larvae, serving for the basis of attachment of powerful muscles which have to do with changes of position of much of the hyoid region, including movements of the lower jaw.

2. Hyobranchial muscles

The hyobranchial muscles of the Spelerpes larva correspond rather closely to those of other larval Urodeles, including such forms as Necturus. They are shown from the ventral aspect in figure 6 and in sections, figures 7 to 16.

The most superficial are the intermandibulares anterior and posterior. These are thin, sheet-like muscles which cover the whole ventral surface of the lower jaw just beneath the skin. The former extends between the two halves of the anterior three-fourths of the mandible; the latter, in its attachments, is rather more complex, as it takes some fibers from the mucous membrane of the pharynx, some from the lateral edge of the distal end of the ceratohyal, some from a fascia which covers the ceratohyoideus externus muscle, and some from the midventral surface of the distal end of the first epibranchial, on both sides.

In young larvae there is but little indication of the median raphé separating the two halves, which is present in metamorphic specimens and adults (see below) as it is in most Urodeles. Out

[5] Stejneger and Barbour ('17) do not include Typhlomolge among the Plethodontidae. The presence of the plate seems to me, however, to be only an additional proof that this animal is in reality a larval Spelerpes, as Miss Emerson so clearly shows ('05).

of about ten more or less complete series of larvae I found a few sections that showed a definite, though narrow raphé (fig. 1, *a*) and a few more, where careful focusing brought out a minute division across the middle of what otherwise appeared to be a single fiber (fig. 1, *b*), but in the majority of cases the fibers were quite unbroken across the midline (fig. 1, *c*).

The geniohyoideus, a long, narrow muscle, arising from the symphysis of the mandible, extends posteriorly at either side of the midline and is inserted into the anterior edge of the lateral projection of the second basibranchial.

The thoracicohyoideus is very large and powerful. Its main portion arises from the pectoral girdle and the whole segmental trunk musculature, passes forward around the pericardial

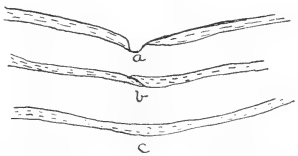

Fig. 1 Representative fibers of the M. intermandibularis drawn from a transverse series.

chamber, and is inserted into the whole dorsal surface of the plate, the posterior end of the first basibranchial and proximal end of the second epibranchial. In addition, a superficial slip arises from a myocomma opposite the proximal end of the third epibranchial, and is inserted into the posterior edge of the lateral projection of the second basibranchial.

The ceratohyoideus externus, the largest of the intrinsic muscles of the hyobranchial apparatus, arises from the middle of the ventral surface of the ceratohyal along almost its entire length, and curves around posterolaterally to be inserted, together with a slip from the dorsal surface of the head, probably the levator arcus primus, into the dorsal surface of the distal end of the first epibranchial.

The ceratohyoideus internus is very much smaller than the externus and is entirely covered by it. It arises from the dorso-

medial surface of the ceratohyal and converges to a narrow insertion on the ventral surface of the proximal end of the first epibranchial.

The adductor arcuum is a ribbon-like muscle which is undoubtedly a fusion of the adductores arcuum secundi et tertii of other forms. It arises from the lateral projection of the second basibranchial, curves around the thoracicohyoideus in a posterolateral direction and divides into two slips, the larger of which is inserted into the ventral surface of the second epibranchial and the smaller into the ventral surface of the third epibranchial.

The two constrictores arcuum (interbranchiales of Drüner) are small muscles, parallel to each other on the gill arches. The first arises from the ventral surface of the first epibranchial, posterior to the insertion of the ceratohyoideus internus, and passes in an oblique posteromedial direction over the insertion of the first slip of the adductor arcuum to be inserted into the ventral surface of the third epibranchial, a little posterior to the insertion of the second slip of the adductor. The second constrictor lies just posterior to the first. It arises from the ventral surface of the second epibranchial and is inserted into the ventral surface of the third.

Five levatores arcuum are represented in the Sperlepes larva, all of which arise from a fascia just beneath the dorsal integument. The first, as mentioned above, is inserted into the first epibranchial along with the ceratohyoideus externus. The second and third are small muscles, inserted into the dorsal surface of the distal ends of the second and third epibranchials, respectively. The fourth (the dorsal half of digastricus pharyngeus of Göppert and dorsobranchialis 4 of Wilder) is a larger muscle, inserted into the tendinous raphé which represents the fourth epibranchial, and the fifth, the dorsotrachealis of lunged forms, is inserted with its fellow in the midline dorsal to the pericardium.

Two depressors arcuum, the third and fourth, occur. The depressor arcus tertius (anterior half of hyotracheales, or pharyngobranchialis 3) arises from the medial border of the third epibranchial and is inserted with its fellow in the midline dorsal to the pericardium. The fourth (posterior half of hyotrachealis,

ventral half of digastricus pharyngeus or pharyngobranchialis 4) arises from the rudimentary fourth epibranchial and is inserted like the third, in the midline. The fifth, like other parts associated with the larynx, is lacking altogether.

ADULT CONDITION

1. Hyobranchial skeleton

In response to its changed functions, the hyobranchial skeleton of the adult has lost almost all resemblance to that of the larva (figs. 17 and 18). From a rather heavy structure with all its parts firmly bound together, it has become a very delicate one with a lack of firm articulations, and thus well adapted to the complexity of motion characteristic of it at this time. This great range of motion makes description rather difficult for the relationships when the tongue is withdrawn are much changed when it is extruded. In the following discussion, I describe the skeleton in the normal resting position with the tongue well withdrawn, unless the contrary is definitely stated.

Though the first basibranchial is still of great importance as supporting the tongue and the cartilages which govern its motion, it is no longer the pivot which binds together all the other parts of the visceral skeleton. Through the loss of the articulation between the ceratohyals and the basal piece and the breaking down of the branchial plate into the first ceratobranchials and the os thyreoideum, the hyobranchial skeleton may be arbitrarily divided into two portions, a central one associated with the first basibranchial and an outlying one, consisting of separate pieces.

Of the latter, the os thyreoideum is the most superficial (fig. 17). This is a small, median, crescentic bone, just beneath the ventral integument, and, despite its minuteness, it is especially significant as the basis of attachment of three important muscles. It is formed by the lateral growth and ossification of the free end of the second basibranchial and is unique as being the only ossified part of the entire hyobranchial apparatus.

The ceratohyal shows perhaps more change from its larval appearance than any other single cartilage. It now lies with its

broad flattened proximal end quite free in the floor of the mouth and projecting slightly into the double fold of mucous membrane dorsal to the tongue stalk (figs. 23 and 24). Posteriorly it becomes narrowed and more rounded, dorsal to the ceratobranchials, till a little posterior to the articulation of the mandible it hooks around, laterally and then anteriorly and has its distal end firmly attached to the quadrate bone.

Of the cartilages of the central portion, the first basibranchial is the pivot, as I have already stated. This median cartilage lies in the tongue stalk and is somewhat curved anterodorsally, with its anterior end in the substance of the tongue (fig. 2). Its ventral surface is flat and, for the anterior two-thirds, is broadened by lateral cartilaginous shelves, formed at metamorphosis. On the

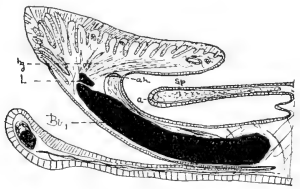

Fig. 2 Median sagittal section of tongue and tongue-stalk of adult.

dorsal surface these shelves help to form rather deep lateral grooves in which the abdominohyoideus muscles lie. The posterior third is more cylindrical and tapers sharply at the tip like the basibranchial of the larva.

A pair of 'little horns,' such as are quite universally present among adult Salamandrids, occurs at the anterior end of the basibranchial. These coruna articulate with the basibranchial anteriorly and extend laterodorsally into the disk of the tongue. Between the horns and dorsal to the basibranchial, is a tiny y-shaped lingual cartilage (figs. 18 and 22) of which I find no mention in any of the description of the visceral skeleton in Plethodontidae. From its position, however, and from its function as the origin of the hyoglossal muscle, it is undoubtedly the morphological equivalent of the 'Sehnen-platte' which Oppel

('00, p. 138) describes in the tongue of Salamandra maculosa and of the 'oto-glossal cartilage' which Cope finds in the Amblystomidae ('87, pp. 87 and 88) and considers a distinctive feature of that family ('89, p. 33).

The first ceratobranchial articulates proximally with the basibranchial at the point where the gradual reduction of the lateral shelf forms an angle in that cartilage; the second ceratobranchial articulates with the posterior end of the basal piece as in the larva (figs. 17 and 18). Both are slender rod-like cartilages which pass posterolaterally to articulate distally with each other and with the proximal end of the first epibranchial. This last-named cartilage is another delicate rod which extends lateroposteriorly and lies at the side of the throat in a curious sac which will be described later under M. ceratohyoideus internus. Only in its anterior third is the adult epibranchial the same cartilage as the larval first epibranchial. Its posterior part consists of a new structure which buds off at metamorphosis, as described below. The distal end of the larval first epibranchial, as well as the whole of the second and third epibranchials, have broken down and become wholly lost.

It may not be extraneous at this point to note the relationships which it is possible for the cartilages of the central portion to assume when the tongue is extruded to its greatest extent. At that time the epibranchials are pushed anteromedially so that their distal ends lie just in the opening of the sac and their proximal ends lie in contact with each other at the curve of the mandible; the ceratobranchials are collapsed like the ribs of a closed umbrella and lie within the epithelium of the tongue stalk with all but their posterior tips entirely outside the mouth; and undoubtedly the 'little horns' are then tipped forward in the plane of the basibranchial as Wiedersheim found to be the case in Salamandrina perspicillata ('75, pp. 88 and 89), though this last fact I have not yet verified.

2. Hyobranchial muscles

The hyobranchial muscles of the adult also show a delicacy and complexity, well adapted to the great mobility of the region. I find in them a marked correspondence, in all but a few comparatively unimportant details, to the muscles of the European species Spelerpes (Geotriton) fuscus, as described in 1875, by Wiedersheim who designated the various muscles merely by letters, as he had not the morphological data to name them. As they also resemble the muscles of the adult Salamandra maculosa described by Drüner in 1904, and as I have traced their development through metamorphosis, I now attempt to give them their morphological names. Figures 19 and 20 show them from more superficial and from deeper ventral dissections, respectively, and figure 21 pictures a pharyngeal (dorsal) dissection. Figures 22 to 27 show important levels in cross-section.

The intermandibularis anterior arises from the inner surface of the mandible and is like the larval muscle, except that the median raphé, which in the larva is scarcely indicated, is now broad and definite.

In the position of the intermandibularis posterior of the larva there appear two distinct muscles. Whether these together are the morphological equivalent of the larval muscle, or whether only the more anterior one represents it, and the more posterior is a new muscle which develops at metamorphosis, I cannot at present state, though the latter view is the more probable. The more anterior of these muscles, the interhyoideus (interossa-quadrata of Drüner) arises from the distal end of the ceratohyal near its attachment to the quadrate bone, but not from the latter bone, as Drüner's name suggests; spreads out like a fan, and is inserted on the median raphé. The more posterior, (Drüner's quadrate-pectoralis) curves around the throat in a posteromedial direction, from just beneath the dorsal integument and is inserted in the gular fold. As I can find in it no relation either to the quadrate bone or to the pectoralis muscle, and as it seems so clearly to be an integumental muscle regulating the gular fold, gularis might be a better name for it.

The adult geniohyoideus consists of two parts, a more superficial geniohyoideus medialis and a deeper one, the geniohyoideus lateralis. The medialis corresponds closely to the entire muscle in the larva. It is a rather broad, extremely thin, ribbon-like band, with its somewhat thicker lateral edge in close association posteriorly with the ceratohyoideus internus which lies just beneath it. As in the larval muscle, its origin is the posterior border of the mandible, lateral to the symphysis, and its insertion, the anterior edge of the os thyreoideum (= distal end second basibranchial). The lateralis arises with the medialis (fig. 22), but lies dorsal and lateral to it, and is inserted along the entire outer border of the ceratohyal. A very thin sheet of fibers from this portion also passes posteriorly dorsal to the hyoid and is inserted in the mucous membrane of the pharynx behind the posterior end of that cartilage (figs. 24 and 25).

The thoracicohyoideus, as such, has broken down, but is still partially represented by the sternohyoideus and the two divisions of the abdominohyoideus. The sternohyoideus is a very thin superficial muscle arising from the dorsal surface of the sternum, and inserted on the posterior border of the os thyreoideum. In its course, it passes latero anteriorly, crossed by two myocommata to a raphé behind the anterior end of the procoracoid, whence it continues medio anteriorly to its insertion. Thus the inner edge of the muscle forms, with that of the other side, a rhomboidal space over the pericardium (fig. 19).

The abdominohyoideus is a very long, round muscle arising on the pelvic girdle and extending forward at the sides of the body, crossed by many myocommata, to a position about behind the most anterior raphé in the sternohyoideus. From this point it sends off a small round slip running anteromedially and inserted on the middorsal surface of the os thyreoideum.[6] The main portion also begins at this point to bend a little medially toward the posterior end of the first basibranchial. There the fibers diverge; some pass straight forward, ventral to the lateral

[6] I find no indication that this slip is continued anteriorly beyond the os thyreoideum as the part of the geniohyoideus medialis which Wiedersheim letters 'f' ('75, fig. 133) and so describes (ibid., pp. 187–8).

edge of the basibranchial, for a short distance, but most of them bend around ventrolaterally and partially enwrap the second ceratobranchial from the ventral side. The muscle then converges again, dorsal to the first ceratobranchial, and, considerably diminished in size, continues forward in the dorsolateral groove of the basibranchial, within the tongue stalk, nearly to the anterior end of the cartilage. Finally it converges dorsolaterally within the tongue substance and inserts through a tendon into the 'little horn.'

Near this lingual insertion of the abdominohyoideus is a muscle, not present in the larva and not very fully developed even in the adult. This is the hyoglossus, which consists merely of delicate fibers which arise from the lingual cartilage and anterior tip of the basibranchial and radiate out through the tongue substance, some to be inserted on the 'little horns' and some in the mucous membrane of the disk.

The ceratohyoideus externus has disappeared entirely, but the simple small ceratohyoideus internus of the larva has become, in the adult, by far the most unique and highly specialized of the entire group. It now arises from the ventral surface of the ceratohyal, and for a short distance its fibers run posteriorly in a straight longitudinal direction. Soon, however, they begin to curve around from the medial edge of the ceratohyal to a position lateral to its outer edge, and back again to a medio-posterio direction. At this point the muscle forms a kind of pocket which opens medially and in which lie the distal ends of the first and second ceratobranchials. Posteriorly, the edges of the pocket converge, and the muscle, added to by new fibers which develop at metamorphosis, becomes wound around the epibranchial in a complicated spiral, the more exact structure of which I have not worked out. The muscle continues a little posterior to the end of the epibranchial, forming a kind of closed sac which, on shortening of the spiral muscle, evidently acts as a bulb and squeezes the epibranchial almost entirely out of the pocket as a means of pushing out the tongue.

Conspicuous in a dorsal view of the floor of the mouth, when the posterior edge of the tongue is lifted forward, even before

the mucous membrane is removed, is an extremely delicate but definitely marked muscle, peculiar to adult free-tongued salamanders, which I call the suprapeduncularis (figs. 21 and 23). It is the muscle which Wiedersheim designates by the letter 'I' ('75, fig. 134) and describes as arising from the medial border of the ceratohyal (ibid., p. 191). I find that in Spelerpes bislineatus, however, it is quite plainly attached to the anterolateral border of the dorsal surface of the ceratohyal on each side and stretches across within the dorsal fold of mucous membrane, with its anterior edge defining and strengthening the free border of the orifice through which the tongue protrudes (fig. 2, *a*).

Of the other larval muscles, most of which were in association with the degenerated epibranchials (constrictors, levators, depressors, etc.), only two are represented. A rather deep-lying stout, muscular band encircles the ventral half of the oesophagus dorsal to the pericardium. The larger part of this is made up by the digastricus-pharyngeus, but a small portion is formed by the dorsolaryngeus. The component parts are not clearly defined however, but tend to form a continuous pharyngeal sheet, as has been pointed out by H. H. Wilder ('96, p. 188).

METAMORPHIC PHENOMENA

1. Skeleton

The metamorphosis of the hyobranchial skeleton of Spelerpes bislineatus shows some very interesting facts which I do not find mentioned in the literature. I have placed these in the form of a normal table which presents the details of development from the larval apparatus to that of the adult. Cross-sections of the four specimens described in this table are pictured in figures 29 to 47, and the condition of the skeleton of a metamorphic animal about like stage III is shown in figure 28.

Perhaps the newest and most striking of the metamorphic phenomena and that which may give a clue to the whole method of skeletal metamorphosis is shown in the development of the adult epibranchial. This cartilage is not merely the larval first epibranchial with its position slightly changed, as has been

generally taken for granted, but it is largely a new structure which has developed from the old in a most curious way. At the stage which Mrs. Wilder calls 'incipient premetamorphic,' when the first signs of approaching metamorphosis appear, I find on the ventral surface of the first epibranchial for a short distance posterior to the insertion of the ceratohyoideus internus and the origin of the constrictor arcuus primi, a slight swelling of the cartilage and conspicuous thickening of the perichrondrium with a number of mitoses (figure 32). This is the anlage of a new portion of the epibranchial. Dorsal to it, a very slight raggedness of the hyaline matrix is the first indication of the degeneration soon to take place. The laying down of new cartilage by the chondrioblasts and degeneration of the old matrix continues slowly until the time when the animal enters into the true 'metamorphic' period. By that time the new sprout has been cut off from the larval first epibranchial for a short distance and appears as a ventral fork of that cartilage with the old matrix degenerating behind it (fig. 36). Posterior to the tip of the new epibranchial, the old one is still quite typically larval. From now on, these phenomena continue rapidly. The new epibranchial grows ventroposteriorly, apparently by proliferation of cells from the perichondrium and frequent mitoses especially near the tip (fig. 3), and the old epibranchial breaks down by an anteroposterior wave of degeneration. The first sign of degeneracy is seen in a raggedness of the haline matrix; then the spaces in the cartilage become filled with leucocytes (phagocytes) which eventually eat away all the old tissue. Thus, by the end of the metamorphic period, the new epibranchial has very nearly its adult proportions and position, and the posterior two-thirds of the larval first epibranchial is almost indistinguishable from a blood-vessel, except for occasional remaining bits of hyaline matrix (figs. 42 and 47).

So, throughout the whole visceral skeleton new pieces develop from old by a proliferation of and subsequent formation of cartilage by chondrioblasts; old ones disappear by a degeneration of matrix and final consumption by phagoctyes, and permanent cartilages change their shape and position by partial degeneration and outgrowth of new tissue in the same ways.

Of the wholly new structures the only examples are the 'little horns' and lingual cartilage. They are no exception to the above rule, but first appear as a collection of chondrioblasts close to the basibranchial—the former, in stage II, and the latter, in stage III (fig. 37)—and later develop hyaline matrix.

Of the cartilages which disappear entirely, the second and third epibranchials are the type. They follow the method of the larval first epibranchial in degenerating in an anteropos-

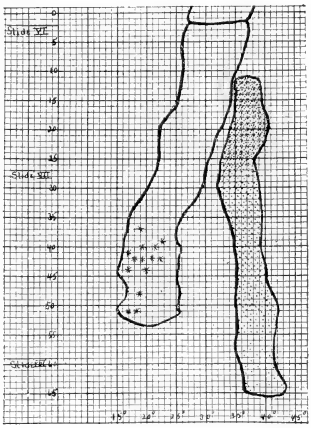

Fig. 3 Millimeter-paper reconstruction of metamorphosing first epibranchial; lateral aspect. Stars indicate location of mitoses. The degree of degeneration of the remaining portion of the old epibranchial is shown by the amount of stippling. × 50.

terior wave. First they lose their connection anteriorly with the rest of the visceral skeleton (fig. 28), and then the disintegration advances more and more posteriorly until they are wholly lost.

In the breaking down of the branchial plate it is interesting to note that the first change comes in a deepening of the groove at which it articulates with the basibranchial, giving the articulation almost an adult appearance as seen in cross-section (figs. 30 and 24), but that the actual degeneration begins in the middle

and cuts off the second basibranchial, and from that position radiates until only the first ceratobranchials and the gradually ossifying osthyreoideum are left.

Almost all of the cartilages show, to some extent, the phenomenon of becoming adapted to their new functions by the principle of degeneration and new growth. In the case of the ceratobranchials and the proximal end of the first epibranchial which is retained through adult life, it is seen in a slight degeneration around the periphery, to give them the slenderness characteristic of the adult skeleton.

The principle is even more marked in the case of the basibranchial, and most evident of all, in that of the ceratohyals. The basal piece, during the metamorphic period, loses its anterior end and attains the flattened ventral surface of the adult by the process of degeneration of old tissue (figs. 34 and 38), and becomes broadened by the lateral shelves which first appear in stage II as thickenings in the perichrondrium, and gradually becomes cartilaginous (figs. 38 and 44). The ceratohyals lose their connection with the basibranchial and assume their more dorsolateral position and adult form by the degeneration of the anterior end, and dorsal, medial, and ventral surfaces anteriorly, and of the whole periphery more posteriorly, and by the outgrowth of new tissue laterally, especially at the posterior end where the 'hook' which becomes attached to the quadrate is almost wholly new.

These processes go on so quickly during the true metamorphic period that at the end of that time all the changes are indicated and only final consumption by phagocytes of the already degenerating matrix, and fuller chondrification of the new parts already laid down, is necessary before the final adult condition is reached.

2. Muscles

The study of the metamorphosis of the hyobranchial muscles shows quite as important results as does that of the transforming skeleton. I have not yet worked out the more minute details of the histological phenomena, but in the main, I find results

similar to those which Mlle. Smirnova ('14) found in the histology of metamorphosing frog muscle. For the order of development of the various muscles from the larval condition to that of the adult and for their appearance in sections I would refer to the same table and plates as in the case of the skeleton.

The general method of muscle metamorphosis is typified in the transformation of the thoracicohyoideus of the larva into the abdominohyoideus and sternohyoideus of the adult. The first indication of any change in the thoracicohyoideus appears in stage I, when a proliferation and anterior growth of fibers from that muscle brings the origin on to the dorsal surface of the first basibranchial, considerably anterior to its articulation with the plate (fig. 30). These new fibers, which are very small and so quite easily distinguishable from the old, make up the anlage of the abdominohyoideus muscle. Stage II shows the origin of what is now quite unmistakably the abdominohyoideus well forward in the connective tissue of the tongue, and the beginning of the breaking down of the median portion of the thoracicohyoideus. In this degenerative process I find, as Mlle. Smirnova did, that the muscle fibers first appear very large and devoid of their cross-striations and then are interspersed with muscle phagocytes which eventually dispose of them. As to the nature of the phagocytes, however, my opinion differs from hers, though I have insufficient data to prove my point conclusively. She states that they are formed by the muscle cells themselves and are not leucocytes, but a careful comparison of them with preparations of fresh human blood shows such a striking similarity to leucocytes of the polynuclear type that I fail to see how they can be anything else. At this stage, also, the differentiation of the ventral slip of the thoracicohyoideus into the sternohyoideus begins by the degeneration of the more dorsal fibers of the slip and ventral ones of the muscle proper (fig. 36). The degeneration of the old and formation of the new fibers continue anteroposteriorly, so that by stage III the abdomino hyoideus is quite adult in position anteriorly, but more posteriorly the whole position of the thoracicohyoideus is filled in with degenerating fibers of that muscle, muscle phago-

cytes, and new fibers of the abdominohyoideus. By stage IV the entire abdominohyoideus and sternohyoideus muscle are quite definitely formed and await only final consumption by the phagocytes of the few remaining degenerate fibers and the filling out to their adult capacity of the new ones already formed.

While the transformation of the thoracicohyoideus into the abdominohyoideus and sternohyoideus involves the two principles—development of new fibers and the degeneration of old—the other muscles exhibit the one principle or the other, and not both. Thus the first alone is found in the geniohyoideus lateralis, which is also present in stage I as a proliferation of cells from the dorsolateral surface of the anterior end of the larval geniohyoideus, or geniohyoideus medialis as it must now be called. In this stage the new fibers are very short and are inserted into the mucous membrane of the pharynx (fig. 29). They now grow rapidly in a posterolateral direction, so that by stage II they approach the ceratohyal; by stage III are inserted on it for a considerable distance, and by stage IV have very nearly their adult insertion. The second principle is found in the adductor, constrictors, first three levators, and the third depressor, which gradually degenerate, as the epibranchials which they regulated break down. The fourth depressor, however, and the fourth and fifth levators, which are hyobranchial muscles only in their comparative morphology, but are physiologically pharyngeal muscles used in respiration, change merely by an increase in size and loss of the identity of their component parts. This last fact brings out quite forcibly the difference between the raphé separating the fourth levator and depressor which has been proved to be a vestigial organ (Göppert, '91, H. H. Wilder, '96), and the raphé separating the moieties of the intermandibularis, which developed secondarily at metamorphosis, evidently in response to an important physiological need, and which is therefore not vestigial, but progressive.

Perhaps the most noticeable and quickly carried out of the changes in the musculature at metamorphosis is furnished by the ceratohyoideus externus and internus. The former, which was by far the largest of the intrinsic larval muscles, but was in

association with the portion of the first epibranchial that breaks down, begins to show sings of degeneracy at stage II; is much reduced in size and very degenerate at stage III, and by stage IV is so far gone as to be almost indistinguishable form a blood-vessel on the ventral surface of the ceratohyoideus internus that lies beneath it (figs. 35, 39 and 46). The latter, which in the larva was small and insignificant, begins at the same time to proliferate new fibers anteriorly and laterally, so that simultaneously with the flattening out of the ceratohyal, the origin of the muscle migrates from the medial border of that cartilage to its whole anteroventral surface (figs. 30, 34, 37, 38, 39, 44 and 45). The muscle also increases in size posteriorly and gradually begins to curve around the ceratobranchials (figs. 35, 40 and 46) to form the adult pocket. The greatest change of all comes at its posterior end where, simultaneously with the outgrowth of the new portion of the epibranchial, there appear muscle fibers encircling the cartilage (fig. 36). These seem to be a proliferation from the end of the ceratohyoideus, as they are perfectly continuous with it; but it is barely possible that they may be developed from undifferentiated mesoderm cells retained in the connective tissue through larval life, and thus not strictly a part of the ceratohyoideus internus. Whatever their origin, they develop very rapidly, always keeping pace with the growing tip of the adult epibranchial, so that by stage III the spiral muscle is fully formed (figs. 41 and 42) and by stage IV it has even begun to be filled out as it is in the adult (fig. 47).

The hyoglossus and suprapeduncularis, the two wholly new adult muscles, are the ones of whose development I am not as yet quite sure. The former appears in stage II as a mass of almost undifferentiated cells, but quite distinguishable from the connective tissue that surrounds them, and in stages III and IV they have assumed the position of, and a slight resemblance to, the radiating fibers of that muscle as they spread out from the anlage of the lingual cartilage. There is no sign of the suprapeduncularis until stage IV, when a few cells, similar to those in the analge of the hyoglossus, appear within the short fold of mucous membrane now formed in the developing tongue-stalk, and undoubtedly represent the anlage of that muscle.

So, with the possible exception of the suprapeduncularis, the same conclusion may be drawn in regard to the hyobranchial muscles as for the hyobranchial skeleton, namely, that during the true metamorphic period all changes are indicated and well on their way to completion, and only a continuation of the process already started is necessary before the adult condition is attained.

CONCLUSIONS

In concluding, there are three points which I wish to emphasize:

First: that the hyobranchial apparatus of the larva is characterized by a certain firmness and rigidity which is greatly increased by the presence of a branchial plate. This plate is a universal phenomenon among larval Salamandrids, but has been generally overlooked in the past.

Second: that the hyobranchial apparatus in the adult has become very delicate and slender, and capable of a great complexity of motion, adapted to its mode of respiration and the capturing of prey.

Third: that the transition from the larval condition to that of the adult is brought about not by a mere breaking down of the parts that are to be lost and the simple shifting of the position of others, but by a complex process which involves the degeneration and loss of certain parts through phagocytosis and the formation of new tissues in the adult position.

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SPECIMEN		CONDITION OF HYOBRANCHIAL SKELETON							CONDITION OF HYOBRANCHIAL MUSCLES						NOTE
Stage and age in relation to origin. Method of preparation	Observations made by Mrs. Wilder at time of killing on condition of gills, respiratory activities	Condition of tongue	Ceratohyals	First basibranchial	Branchial plate, ceratobranchials, osthyreoideum	Epibranchials	Little horns, lingual cartilage	Intermandibulares	Geniohyoideus	Thoracirhyoideus (abdominohyoideus sternohyoideus)	Ceratohyoideus externus and internus	Adductor, constrictors depressores, leratores	Hyoglossus suprapedunculars	
Stage I *1914A* Killed Worcester-Wilder Fixative, June 28, 1914 Sectioned, 15 μ trans. November, 1917 Stained, haematoxylin		Larval	Larval	Larval	Plate quite larval, though anterior groove rather deep and median portion rather thin Os thyreoideum beginning to ossify	Anlage of adult eb_1 present as slight swelling of cartilage and thickening of perichondrium with frequent mitoses, at level of insertion of *M.ch*i and origin of *M.ca* and for about 600 μ posteriorly		Median raphé formed in *M.imx* except for the few most anterior sections. Raphé formed in *M. imp.* anteriorly. Larval posteriorly	*M.ph* budded off from *M.ghm* anteriorly for about 800 μ. Inserts into mucous membrane of pharynx	Origin on dorsal surface of *Bb*, considerably anterior to articulation of plate with it	Larval	Larval		Specimen corresponds to Mrs. Wilder's stage 'Incipient premetamorphic' *1916D*$_1$ was in same condition. *1914D* somewhat more advanced
Stage II *1914C* Killed Worcester-Wilder Fixative, June 28, 1914 Section, 15 μ trans. November, 1917 Stained, haematoxylin	Gills still have flow of blood in them Lifting head out of water and breathing air	Folds in mucous membrane of floor of mouth, including larval tongue, straightened out Anlage of adult tongue longitudinal outfolding of mucous membrane with anterior end glandular	Articulation with basibranchial breaking down. Ventral surface *slightly*; proximal end, dorsal and mediodorsal surfaces *rapidly*, degenerating. Posterior 'hook' budding off laterally as new cartilage	Anterior end degenerating rapidly. Ventral surface beginning to degenerate Anlage of lateral shelves present as collection of chondroblasts	Plate degenerating in the middle Cb_1 degenerating around periphery anteriorly Osthyreoideum somewhat more ossified	Larval epibranchials degenerating around periphery anteriorly Adult eb_1 formed as ventral fork of cartilage for about 750 μ. Dorsal to it, larval eb_1 degenerating. Posterior to it, eb_1 shows slight degeneracy and eb_2 and eb_3 are quite larval	Anlage of horns present as collection of chrondrioblasts	Median raphé in both *M.imx* and *M.imp.*	*M.gh* more differentiated from *M.ghm*, though still small, and entered into pharyngeal wall. Approaching ceratohyals, however	Origin of *M.ah* in connective tissue of tongue posterior to anlage of horns. *M.th* beginning to degenerate medially *Sternohyoideus* becoming differentiated from *M.ths*	*M.che* beginning to degenerate, especially posteriorly. Insertion larval *M.chi* forming new fibers and increasing in size. Origin more anterior and ventral. Growing posteriorly and becoming curved around $Cb_{1,2}$ and Eb_1. Spiral fibers forming around new eb_1	*M.aa* degenerating slightly *M.ca*$_1$ degnerating. Others larval	Anlage of hyoglossus present as almost undifferentiated cells	Probably early 'metamorphic'
Stage III *1915 J*$_1$ $c = 60 +$ mm. Killed Worcester-Wilder Fixative, July 3, 1915 Sectioned, 20μ trans. November, 1917 Stained, haematoxylin and eosin	Gills: Pale, bushy, but not curved forward. Beaten muscularly, slightly and irregularly Right gill reduced	Outfolding in mouth beginning to assume shape of disk Quite glandular	Articulation with basibranchial gone. Becoming somewhat flattened Considerable degeneration still taking place	Old anterior end (anterior to horns) represented by leucocytes and shreds of cartilage Ventral surface becoming flattened. 'Shelves' becoming cartilaginous	Plate broken down, but first ceratobranchials still in ventral plane with many leucocytes and suggestion of perichrondrium between them Osthyreoideum more ossified	Adult epibranchial formed for about 1200 μ. Larval eb_1 very degenerate. Consists mainly of leucocytes and shreds of cartilage anteriorly and degenerating cartilage posteriorly. Eb_3, degenerating rapidly. Very ragged with many leucocytes, especially anteriorly	Horns becoming chondrified. Anlage of lingual cartilage present	Median raphé formed throughout Interbyoideus and gularis becoming separate	*M.ghl* inserted on gh for considerable distance	*M.ah* adult anteriorly. Posteriorly, large number of leucocytes, degenerating fibers of *M.th* and new fibers of *M.ah* fill old position of *M.th* *M.sh* more differentiated	*M.che* still present but very degenerate. Origin on *M.chi* (not *Ch*) *M.chi* origin anterior and ventral. Assuming adult position and shape and spiral formed but not filled out	*M.aa* degenerating *M.ca*$_{1,2}$ nearly gone *M.Pa*$_{1-3}$ degenerating *M.da*$_1$ degenerating	Anlage *M.hg* assuming radiating position	Probably late 'metamorphic,' approaching advanced metamorphic. Not very different from *1914B*$_1$. In condition of gills and visceral skeleton, younger and in condition of skin, glands, dermal bones, naso-lachrimal duct, head shape, etc., older than B_1 Leucocytes emphasized by eosin of stain
Stage IV *1914 B*$_1$ c = circum 55 mm. Killed Worcester-Wilder Fixative, June 28, 1914 Sectioned, 15μ trans. December, 1917 Stained, haematoxylin	Gills large and very red. Blood moving steadily through them. Crawls out of water somewhat	Disk about formed. Beginning of infolding of pharynx beneath it to form stalk	Articulation with basibranchial quite gone. Anterior end still degenerating. Becoming quite flattened, though dorsal and ventral degeneration still going on Posteriorly degenerating all around periphery	Only a faint suggestion of former anterior end left Ventral surface quite flat though old outline and leucocytes present 'Shelves' about as in *1916 J*$_3$	Plate almost broken down but a few shreds of cartilage mark its former position Osthyreoideum more ossified	Adult epibranchial formed for about 1350 μ. Larval eb_1 practically gone. Position marked by collection of leucocytes with occasional shreds of cartilage	About as in *1916 J*$_3$	About as in *1916 J*$_3$	*M.ghp* inserted on ch for its anterior half. Fibers still small	*M.ah* practically adult anterior to osthyreoideum. Fibers small and some degenerate fibers of *M.th.* left. *M.sh* about as in *1916 J*$_3$	*M.che* practically gone *M.chi* has almost adult form but not fully developed, though more filled out than in *1916 J*$_3$	*M.ac* degenerate *M.ca*$_{1,2}$ nearly gone *M.la*$_{1-3}$ gone *M.da*$_1$ degenerate	Anlage *M.hg* about as in J_3 Anlage *M.sp* forming?	Probably late 'Metamorphic' *1914IV*, *1917C17* and *1915 J*$_4$ were in same condition

PLATES

The outlines in plates 1, 5, and 10 were drawn from methylen-blue preparations with the His embryograph and enlarged with pantograph. In finishing, certain details were added from reconstruction of sections. Plates 6, 7, and 8 were drawn free-hand from dissections with the camera outlines of plate 4 as the skeletal basis. The outlines of all the cross-sections were made with the projection lantern.

ABBREVIATIONS

Skeleton

Bb 1–2, basibranchial 1–2
Bp, branchial plate
Cb 1–2, Ceratobranchial 1–2
Ch, ceratohyal
Eb 1–3, epibranchial 1–3
*Eb**, developing new portion of first epibranchial
H, little horn
*H**, anlage, little horn
L, lingual cartilage
*L**, anlage, lingual cartilage
Ot, os thyreoideum

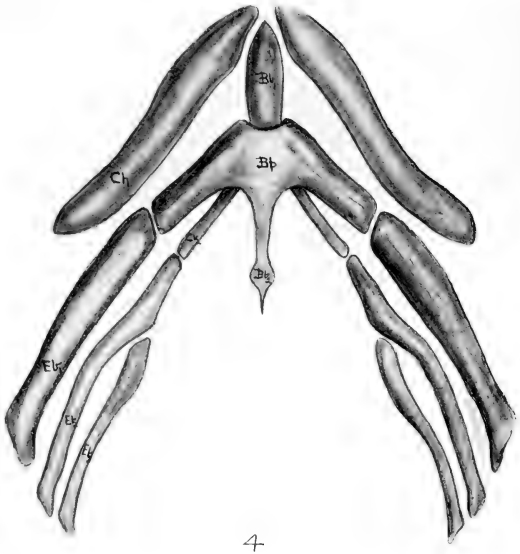
Muscles

aa, adductor arcuum
ah, abdominohyoideus
*ah**, developing abdominothyoideus
ahs, ventral slip of abdominohyoideus
ca 1–2, constrictores arcuum 1–2
che, ceratohyoideus externus
chi, ceratohyoideus internus
da 3–4, depressores arcuum 3–4
g, gularis
gh, geniohyoideus
gh l, geniohyoideus lateralis
gh m, geniohyoideus medialis
hg, hyoglossus
*hg**, anlage hyoglossus
ih, interhyoideus
ima, intermandibularis anterior
imp, intermandibularis posterior
la, 1–5, levatores arcuum 1–5
Sh, sternohyoideus
Sp, suprapeducularis
th, thoracicohyoideus
ths, ventral slip of thoracicohyoideus

PLATE 1

EXPLANATION OF FIGURES

4 Hyobranchial skeleton of larva; ventral view. $\times 37\frac{1}{2}$.
5 Hyobranchial skeleton of larva; dorsal view. $\times 37\frac{1}{2}$.



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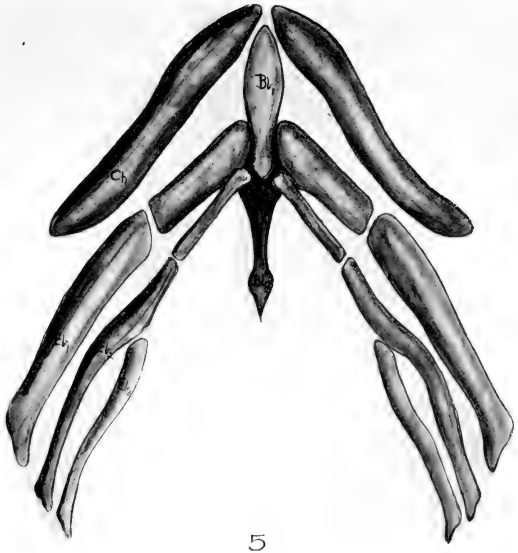


5

PLATE 2

EXPLANATION OF FIGURE

6 Hyobranchial muscles of larva; ventral view. The lines 7 to 16 show the levels at which the sections shown in plates 3 and 4, figures 7 to 16, respectively, were cut. ×50.

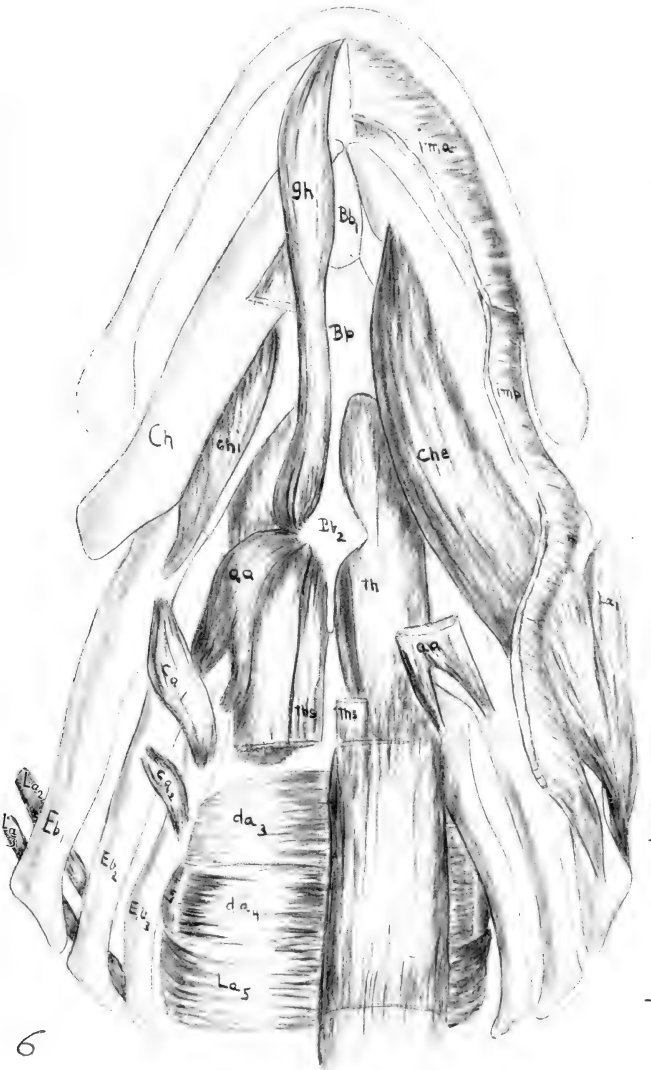

gh
Bb1
Bp
Ch
chi
Che
Bv2
qa
th
Cq1
Cq2
ths
da3
da4
La5
Eb1
Eb2
Eb3
Lm
Lp
7
8
9
10
11
12
13
14
15
16
6

PLATE 3

EXPLANATION OF FIGURES

Cross-sections of larva. ×40

7 Level of articulation of ceratohyals with basibranchial 1.

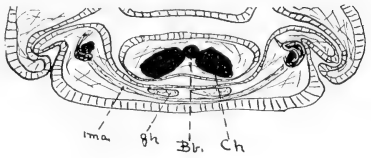
8 Level of the origin of M. ceratohyoideus externus.

9 Level of the origin of M. ceratohyoideus internus, showing the plate near its anterior end.

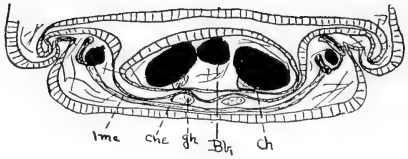
10 Level of the origin of M. thoracicohyoideus, showing plate near its posterior end.

11 Level showing M. thoracicohyoideus at level of posterior ends of ceratobranchials.

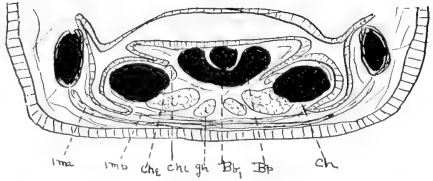
12 Level of insertion of M. geniohyoideus.


Ima
gh
Br.
Ch

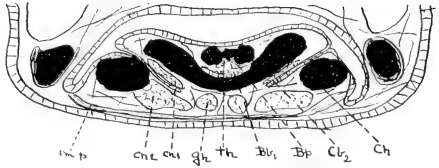
7


Ime
che
gh
Br1
Ch

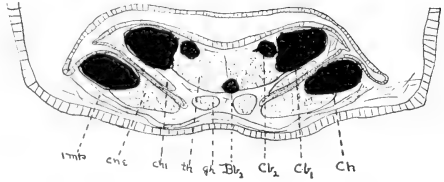
8


Ima
Imb
Che
Chi
gh
Br1
Bp
Ch

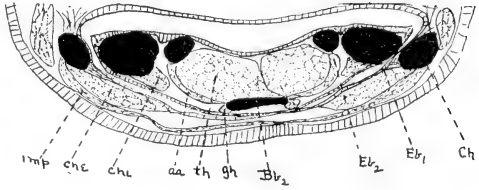
9


Imp
che
chi
gh
th
Br1
Bp
Cb2
Ch

10


Imb
che
chi
th
gh
Br3
Cb2
Cb1
Ch

11


Imp
che
chi
aa
th
gh
Br2
Eb2
Eb1
Ch

12

PLATE 4

EXPLANATION OF FIGURES

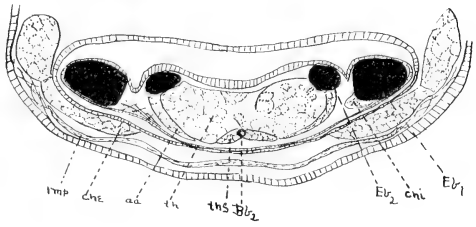
Cross-sections of larva. × 40

13 Level of insertion of M. ceratohyoideus internus, showing slip of M. thoracicohyoideus, and M. adductor arcuum.

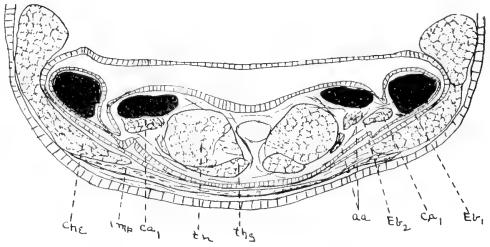
14 Level showing M. constrictor arcuum 1.

15 Level of insertion of ceratohyoideus externus, showing M. depressor arcuum 3.

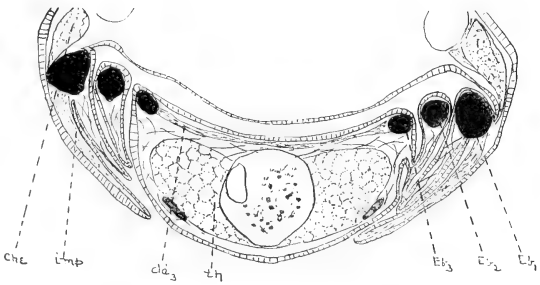
16 Level showing M. levatores 3 and 5.


imp
chε
ad
th
ths
Bb2
Eb2
chi
Eb1

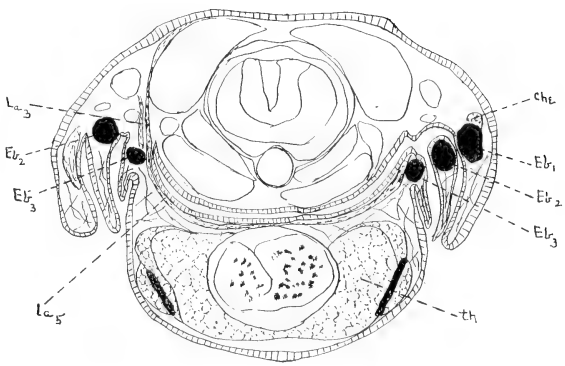
13


chε
imp
ca1
th
ths
aa
Eb2
ca1
Eb1

14


chε
imp
cla3
th
Eb3
Eb2
Eb1

15

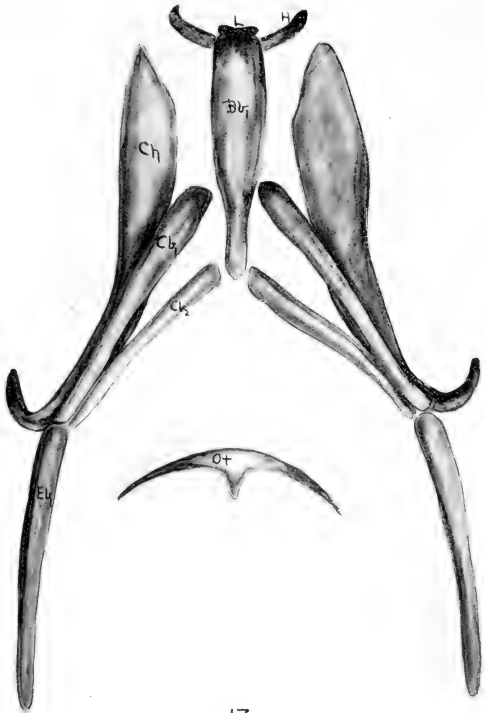

la3
Eb2
Eb3
la5
chε
Eb1
Eb2
Eb3
th

16

PLATE 5

EXPLANATION OF FIGURES

17 Hyobranchial skeleton of adult; ventral view. ×20.
18 Hyobranchial skeleton of adult; dorsal view. ×20.



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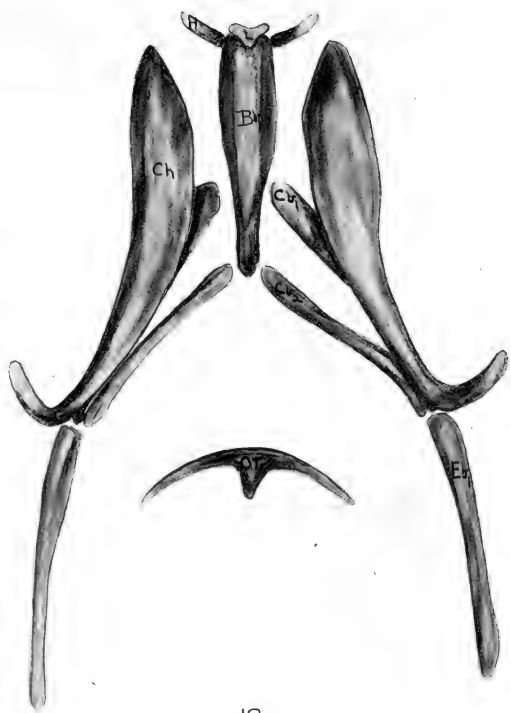


18

PLATE 6

EXPLANATION OF FIGURE

19 Superficial dissection of hyobranchial muscles of adult; ventral view. ×20.

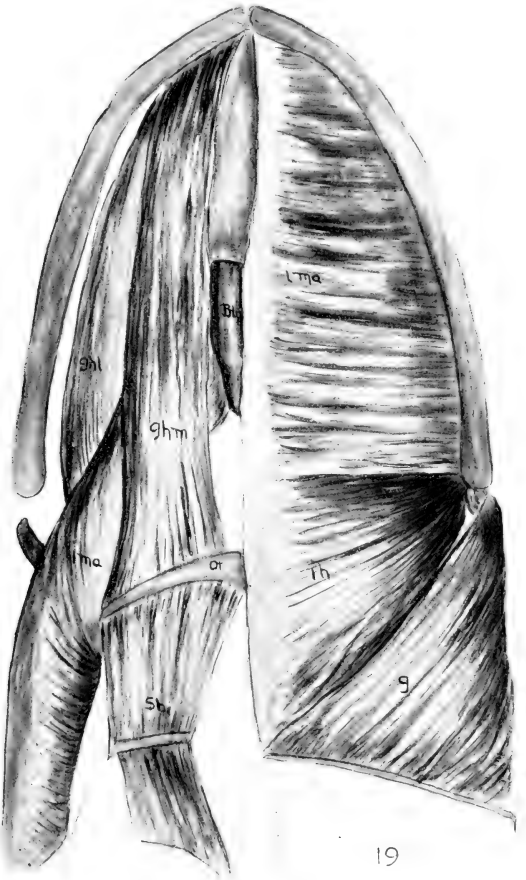

ghl
ghm
tma
or
sb
tma
th
g
19

PLATE 7

EXPLANATION OF FIGURE

20 Deeper dissection of hyobranchial muscles of adult; ventral view. The mucous membrane of the floor of the mouth has been partially removed to show its relationship to the tongue and tongue-stalk. ×20.

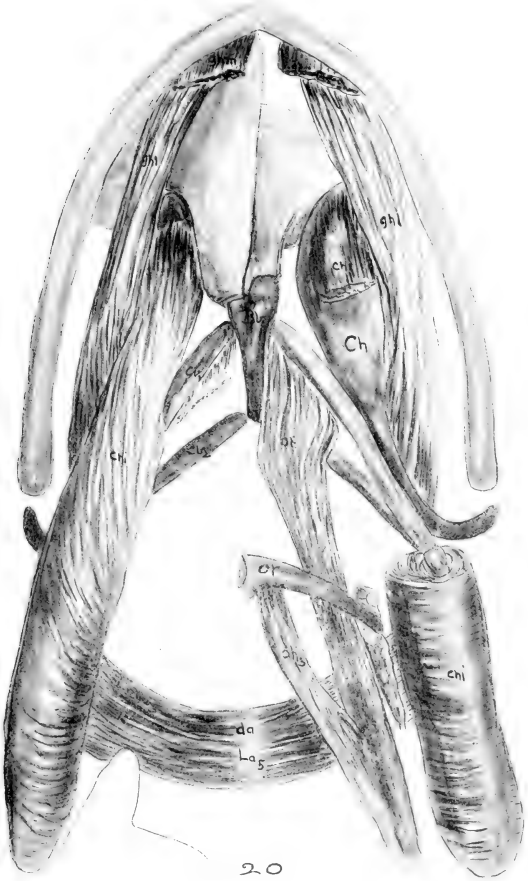
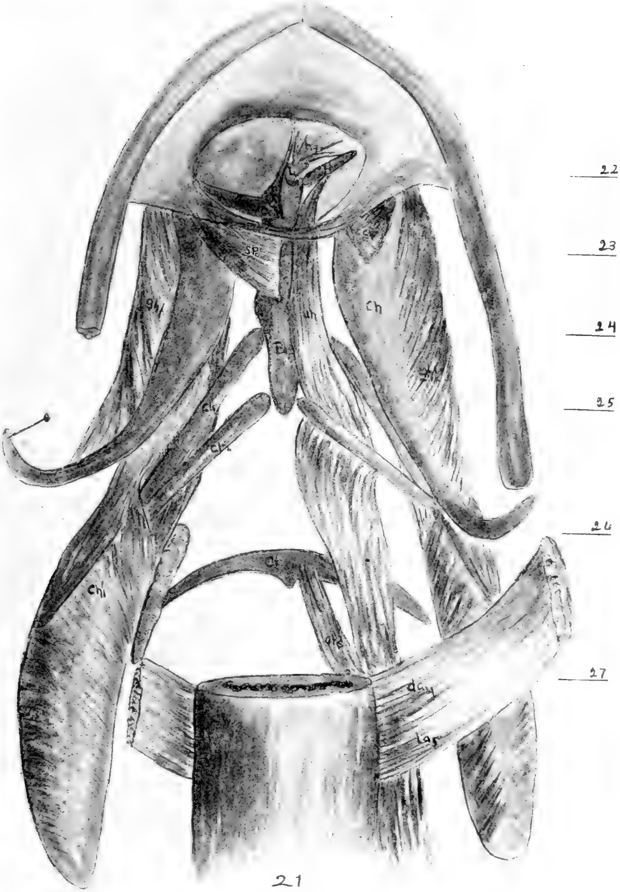

ghi
ghi
ghi
Ch
Ch
Ch
cr
cr
chi
chi
da
20

PLATE 8

EXPLANATION OF FIGURE

21 Dissection of hyobranchial muscles of adult; dorsal view. The dorsal surface of the tongue has been partially removed, and the posterior edge entirely so, to show underlying structures. The lines 22 to 27 show approximately the levels at which the sections shown in figures 22 to 27, respectively, were cut. These levels cannot be shown exactly, as the position of the apparatus at the time of fixation was different in the two specimens. ×20.



21

PLATE 9

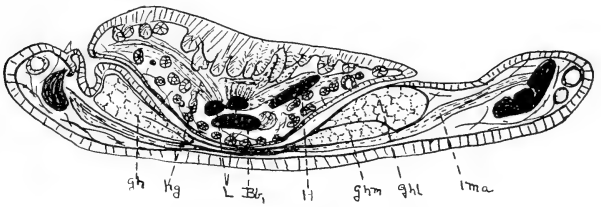
EXPLANATION OF FIGURES

Cross-sections of the adult. ×28

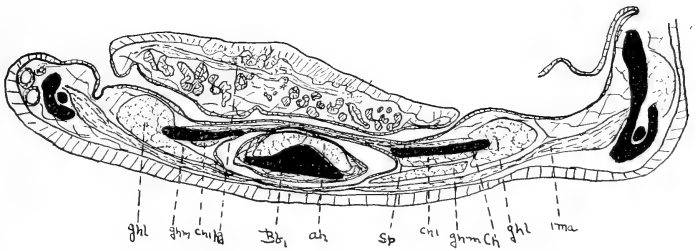
22 Level of anterior end of first basibranchial, showing lingual cartilage and 'horn' in tongue.

23 Level of the origin of M. ceratohyoideus internus, showing M. suprapedunecularis and the tongue stalk.

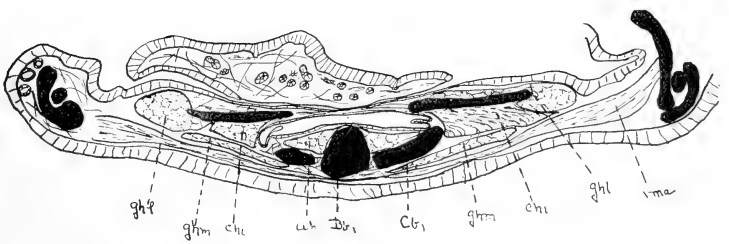
24 Level of articulation of the first ceratobranchials with basibranchial.


gh
Hg
L
Br1
Hy
ghm
ghl
Ima

22


ghl
ghm
chl
Hg
Br1
ah
Sp
chl
ghm
Ch
ghl
Ima

23


ghl
ghm
chl
ah
Br1
Cb1
ghm
chl
ghl
Ima

24

PLATE 10

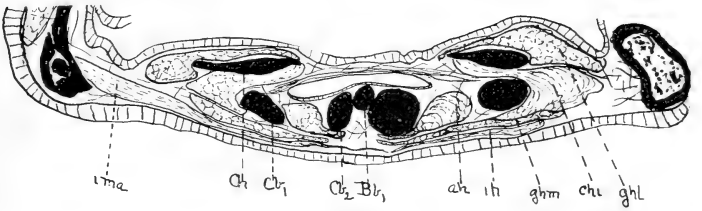
EXPLANATION OF FIGURES

Cross-sections of adult. × 28

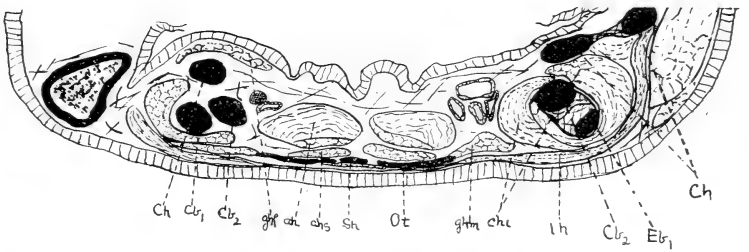
25 Level of articulation of second cerabranchials with basibranchials, showing the beginning of the pocket formed by the ceratohyoideus internus muscle.

26 Level of the os thyreoideum and of the articulation of the epibranchial with the first and second ceratobranchials, where the ceratohyoideus internus begins to form the spiral.

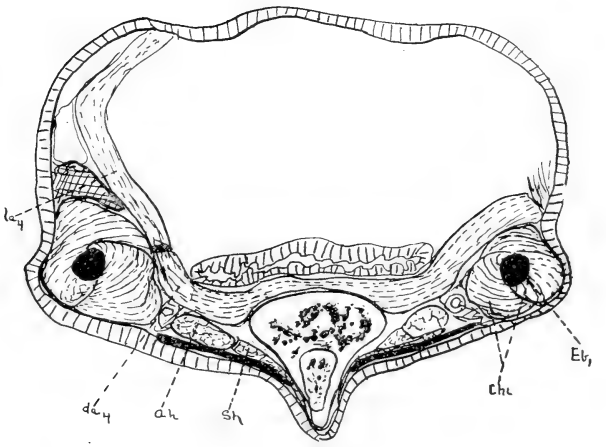
27 Level of the heart, showing the fourth levator and depressor of the gill arches, and the spiral muscle toward the posterior region of the epibranchial.


ima
Ch
Br1
Br2
ah
ih
ghm
chi
ghl

25


Ch
Br1
Br2
ghl
ah
Sh
Ot
ghm
chi
ih
Br2
Br1
Ch

26


Br4
dBr4
ah
Sh
chi
Br1

27

PLATE 11

EXPLANATION OF FIGURE

28 Hyobranchial skeleton of metamorphic stage III, ventral view. The position of cross-sections cannot be shown on this drawing, as the specimen of stage III, used for sectioning, differed somewhat in development and considerably in position of cartilages from this one. ×27.

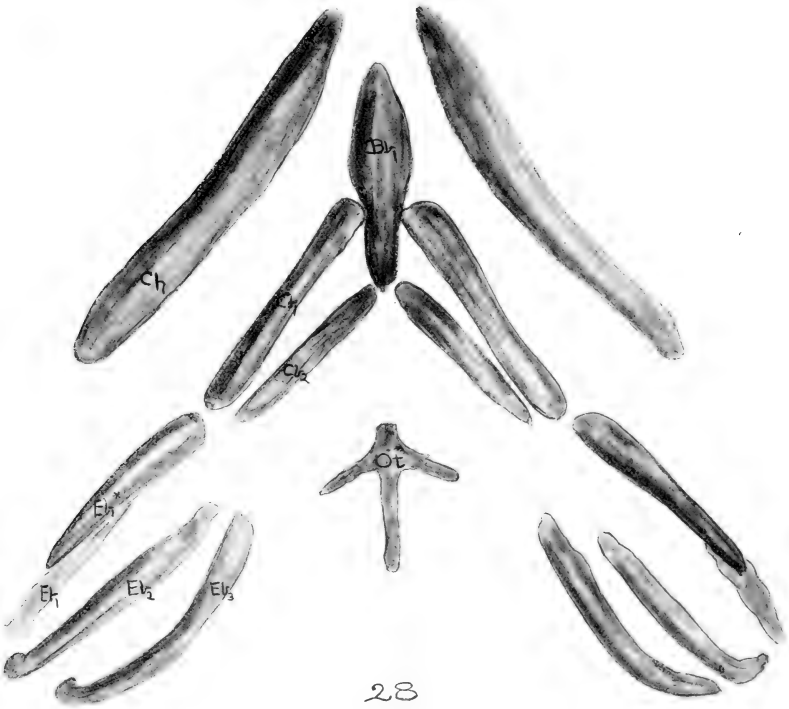

Bh1
Ch
Ch1
Ch2
Ot
Eh1'
Eh1
Eh2
Eh3
28

PLATE 12

EXPLANATION OF FIGURES

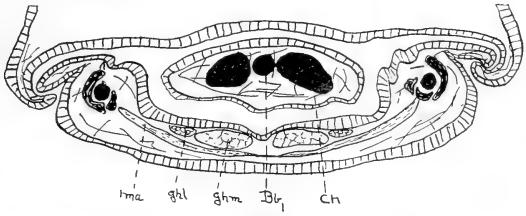
Cross-sections of metamorphic stage I. ×28

29 Level corresponding to larva, figure 7, showing presence of M. geniohyoideus lateralis anteriorly.

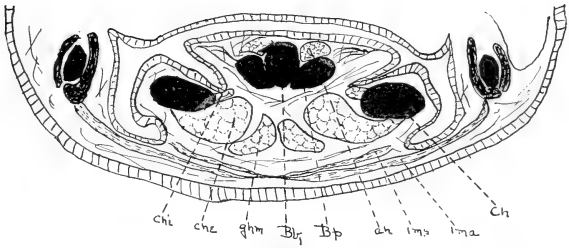
30 Level corresponding to larva, figure 9, showing the deepening of the groove at which the branchial plate articulates with the basibranchial, and the anterior growth of the abdominohyoideus muscle.

31 Level corresponding to larva, figure 10, showing development of the abdominohyoideus, and absence of change in other particulars.

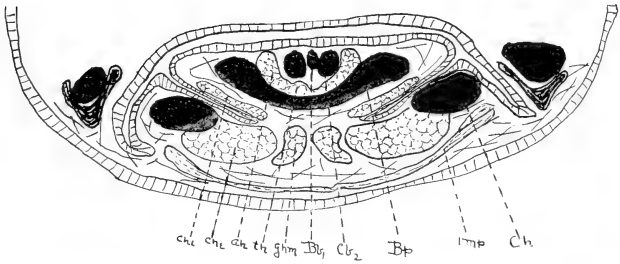
32 Level corresponding to larva, figure 14, showing the anlage of the adult epibranchial and beginning of degeneration of the larval first epibranchial.


ima
ghl
ghm
Bl1
Ch

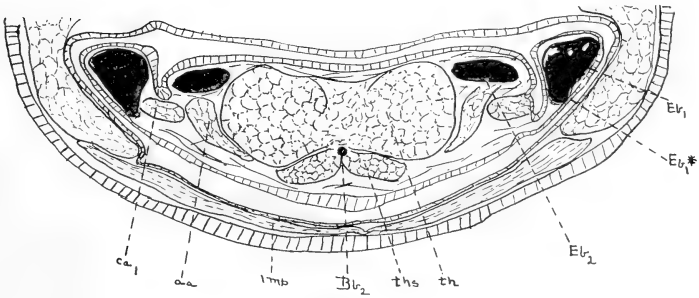
29


chl
chc
ghm
Bl1
Bp
dh
ims
ima
Ch

30


chl
chc
dh
th
ghm
Bl1
Cb2
Bp
imp
Ch

31


Eb1
Eb1*
Eb2
ca1
aa
imb
Bl2
ths
th

32

PLATE 13

EXPLANATION OF FIGURES

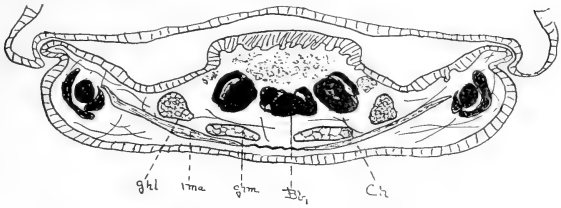
Cross-sections of metamorphic stage II. ×28

33 Level corresponding to a section of larva between figures 7 and 8, showing degeneration of anterior end of basibranchial and ceratohyals.

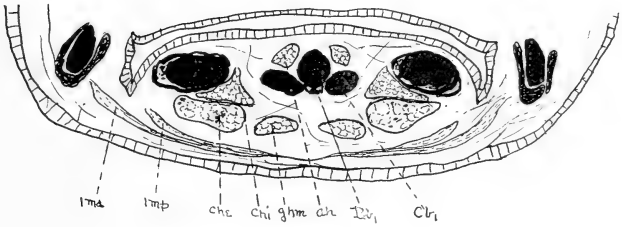
34 Level corresponding to larva, figure 9, and metamorphic stage I, figure 30, showing degeneration of basibranchial, ceratohyals, and M. ceratohyoideus internus, and anterior growth of Mm. abdominohyoideus and ceratohyoideus internus.

35 Level corresponding to larva, figure 10, showing degeneration of the plate and of the median portion of M. thoracicohyoideus.

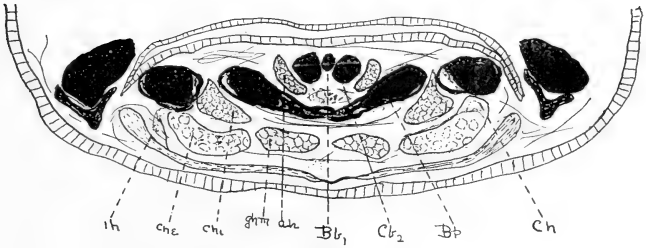
36 Level corresponding to metamorphic stage I, showing further growth of adult epibranchial, anlage of spiral muscle and degeneration of larval first epibranchial.


ghl
ima
ghm
Br1
Ch

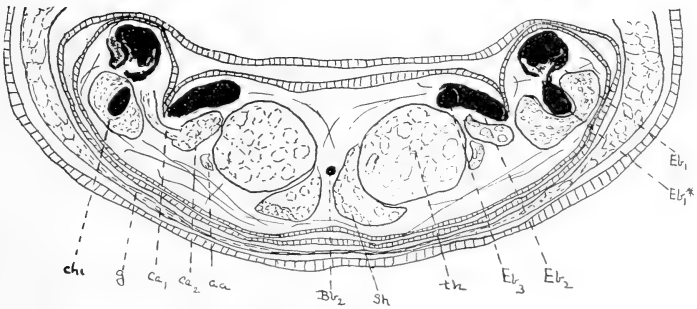
33


ima
imp
che
chi
ghm
dh
Br1
Ch1

34


ih
che
chi
ghm
dh
Br1
Ch2
Bp
Ch

35


chi
g
ca1
ca2
aa
Br2
sh
th
Br3
Br2
Br1
Br1*

36

PLATE 14

EXPLANATION OF FIGURES

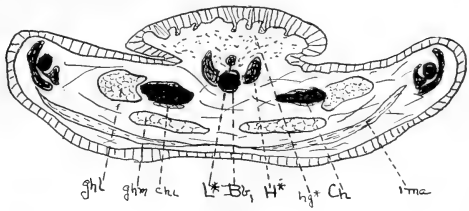
Cross-section of metamorphic stage III. ×28

37 Level corresponding to adult, figure 22, showing anlage of 'little horns' and lingual cartilage.

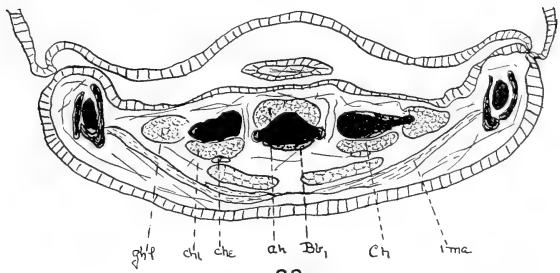
38 Level corresponding to adult, figure 23, showing changing shape of basibranchial and ceratohyals.

39 Level corresponding to levels shown in larva figure 9, in metamorphic stages II and III, figures 30, 34, and in adult figure 24.

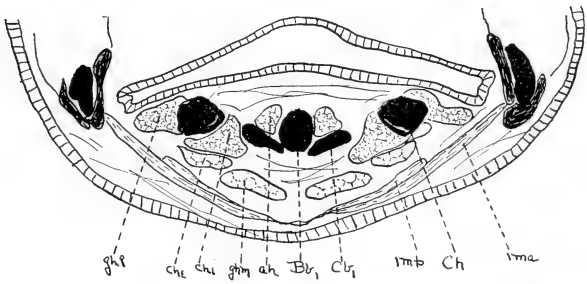
40 Level corresponding to larva, figure 10, and adult, figure 25, showing the plate and M. thoracohyoideus nearly broken down.


ghl
ghm
chi
L*
Br1
H*
hy*
Ch
ima

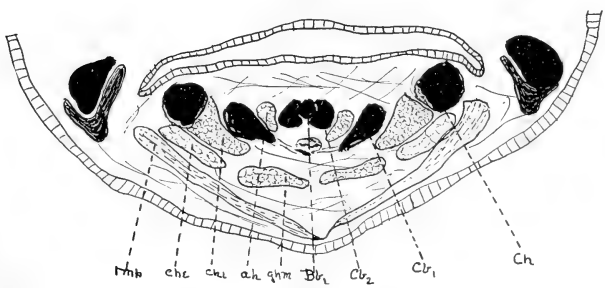
37


ghp
chi
che
ah
Br1
Ch
ima

38


ghp
che
chi
ghm
ah
Br1
Cb1
imb
Ch
ima

39


imb
che
chi
ah
ghm
Br2
Cb2
Cb1
Ch

40

PLATE 15

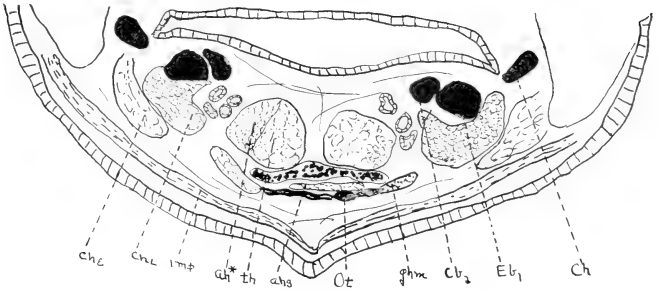
EXPLANATION OF FIGURES

Cross-sections of metamorphic stage III. × 28

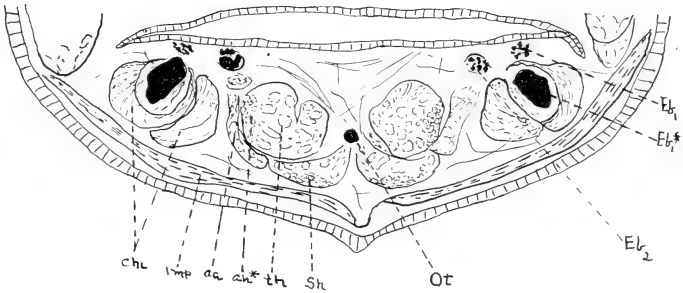
41 Level corresponding to larva, figure 12, and adult, figure 26, showing the rapidly ossifying os thyreoideum, the M. thoracicohyoideus degenerating and M. abdominohyoideus developing, and the M. ceratohyoideus internus beginning to form the spiral.

42 Level corresponding to larva, figure 14, and metamorphic stage II, figure 36, showing the further development of the adult epibranchial and spiral muscle, and the almost complete degeneration of the larval first and second epibranchials.

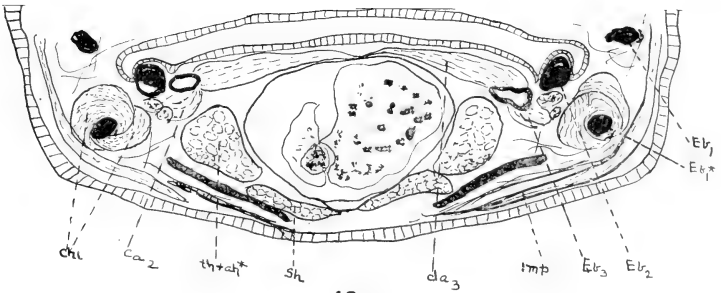
43 Level corresponding to larva, figure 15, showing the M. sternohyoideus completely separated from the thoracicohyoideus and the adult epibranchial and spiral muscle formed, the posterior ends of the larval epibranchials still present.


ch_c
ch_c
Imp
ah*
th
ahg
Ot
ghm
Cb_2
Eb_1
Ch

41


Eb_1
Eb_1*
Eb_2
ch_c
Imp
aa
ah*
th
Sh
Ot

42


Eb_1
Eb_1*
ch_c
Ca_2
th+ah*
Sh
da_3
Imp
Eb_3
Eb_2

43

PLATE 16

EXPLANATION OF FIGURES

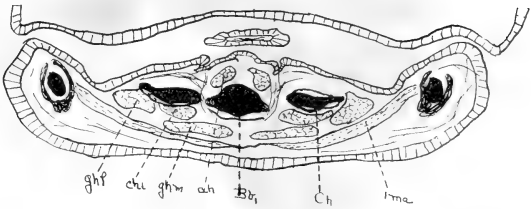
Cross-sections of metamorphic stage IV. ×28

44 Level corresponding to metamorphic stage III, figure 38, showing the infolding of the pharyngeal mucous mmbrane to form the tongue stalk, the further flattening out of the basibranchial, and ceratohyals, and the complete loss of the M. ceratohyoideus externus anteriorly.

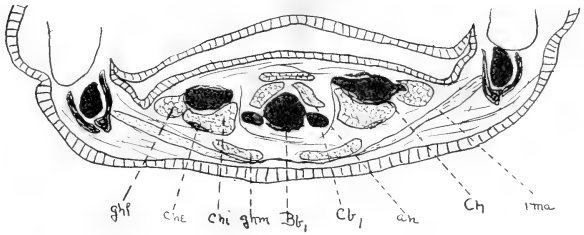
45 Level corresponding to adult, figure 24, showing posterior limit of formation of tongue stalk.

46 Level corresponding to metamorphic stage III, figure 40, showing nearly complete degeneration of M. ceratohyoideus externus.

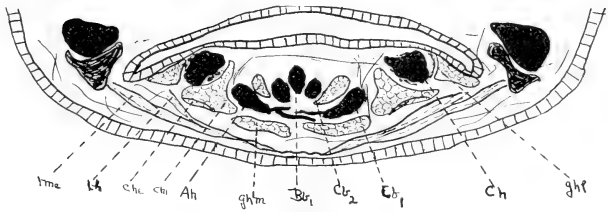
47 Level corresponding to metamorphic stage III, figure 42, showing the spiral muscle beginning to fill out to its adult proportions, and the M. thoracicohyoideus nearly transformed into Mm. abdominohyoideus and sternohyoideus.


ghf
chi
ghm
ah
Br1
Ch
Ima

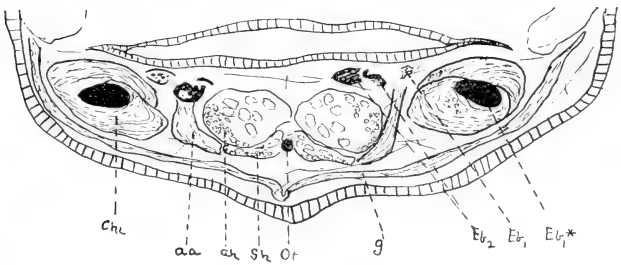
44


ghf
chi
ghm
Br1
Cb1
Ch
Ima

45


Ima
Ah
ghm
Br1
Cb2
Cb1
Ch
ghf

46


chi
aa
ah
Sh
Ot
g
Eb2
Eb1
Eb1*

47

SUBJECT AND AUTHOR INDEX